The Journal of Parasitology

Volume 4 SEPTEMBER, 1917 Number 1

ON THE STRUCTURE AND CLASSIFICATION OF NORTH AMERICAN PARASITIC WORMS*

HENRY B. WARD

For many years I have been engaged in the study of parasitic worms from North American freshwater hosts, mostly fish; during this time I have had opportunity to examine and compare material from a large number of localities embracing many widely separated points. In this work I have been aided very greatly by studies on individual groups undertaken and published under my direction by various graduate students to whom my obligation is freely expressed here. In connection with this work it has been necessary to examine critically all original records of parasites from similar hosts and to endeavor to reach a positive determination of the parasite under discussion in each case. This is not a simple matter, as the records were often made on the basis of a rapid preliminary examination; furthermore, the total lack of special reference works on these groups led to the recording of parasitic species under general names taken from the older European writers, and these names are often wanting in definite significance.

My work has naturally led to the discovery of new facts regarding the structure of the forms studied and has compelled me to introduce new names and to rearrange forms so as to express better their correct relationships in the light of more perfect knowledge of their structure. Such changes are of course unfortunate in that they make it difficult to trace the continuity between the new and the old in zoological literature; they are nonetheless essential if the student is to apprehend the true character and affiliations of the forms with which he comes in contact. It has been my fixed principle never to make any changes until I was personally familiar with the form discussed or had acquired such acquaintance with its structure as to know that some change was inevitable and that the proposed modification was defensible on morphological grounds. Most of the questions involved in the changes listed later in this paper have been submitted to the criticism of advanced workers in the field, or discussed before graduate classes for some years so that they may be regarded as seasoned changes.

* Contributions from the Zoological Laboratory of the University of Illinois, No. 94.

Some of the more general results of my work have been included in brief form in synopses of the Parasitic Flatworms and Parasitic Roundworms which constitute two chapters in Freshwater Biology by Ward and Whipple just being published by John Wiley and Sons, New York. It seems to me wise to print here in outline the most important new material regarding the structure and classification of the parasitic forms discussed in these chapters as there are items which might easily escape notice and thus lead to confusion if published only in a text-book. The work just cited gives complete summaries of the North American forms in the groups mentioned and the place of the items discussed later in this paper may be precisely determined by reference to it. These items are arranged here in the order in which they are taken up in the book, and this is the systematic arrangement. The student will also find there an abundance of illustrations to demonstrate the points discussed here.

TAXONOMIC CHANGES AMONG TREMATODA

The genus Polystoma established by Zeder in 1800 is well known thru the common European type, *P. integerrimum,* generally employed in text books to illustrate the group of monogenetic termatodes. Several species from North America have been carefully described by Stunkard (1917). These forms stand out distinctly when compared with the European type and clearly constitute a separate section of the genus to which I have given the name Polystomoides. This subgenus which further study may show to be of generic rank is characterized by the presence of a short uterus containing only a single egg whereas the European type possessed a uterus of several coils with numerous eggs. Stunkard has also pointed out considerable differences in the structure of the suckers on the caudal disc. As type may be designated P. (*Polystomoides*) *coronatum* (Leidy 1888) from the common food terrapin.

As the cause of an epidemic among sparrows at Madison, Wisconsin, Cole (1911) reported under the name of *Monostoma faba* a trematode that in reality differs distinctly from the European species. The form of the ovary, the extent of the vitellaria, the dermal spines, and other details of structure disagree with the recent description of Kossack who moreover assigned Rudolphi's species to his new genus Collyriclum. The American form constitutes a new species in this genus and to it the name *Collyriclum colei* may be given. The position of this genus is so isolated among the monostomes that a new family must be created for it. This may be characterized as follows:

COLLYRICLIDAE Ward. Small to moderate sized monostomes with discoidal, compressed, not muscular body, broader than long. Oral sucker weak; pharynx present; ceca simple, long, capacious, not united. Genital pore ventral, near center of body. Vitellaria follicular, scanty, antero-lateral; ovary much lobed, asymmetrical. Uterus posterior, in irregular coils which show an antero-posterior tendency, terminal region enlarged. Testes oval, symmetrical, behind ovary. Eggs very small. Adults parasitic in dermal cysts on abdominal surface of skin in birds.

What is probably the same species has also been found parasitic on sparrows at Boston, Mass. Its appearance is concurrent with periods of wet weather.

In 1902 MacCallum described an interesting parasite from the lungs and air passages of the river snapping turtle (*Chelydra serpentina*) found in Ontario, Canada. To this he gave the name of *Heronimus chelydrae*. In 1914 Barker and Parsons described a very similar form from the lungs of *Chrysemys marginata* taken in Minnesota, and also of various other turtles from Nebraska. This parasite they named *Aorchis extensus*. I have collected specimens from Illinois, Indiana, and Michigan of what is probably the same species. These two forms are so much alike that they may prove to be identical or at least to belong to the same genus, but they are in some respects very different from any other monostomes known, and I have established for them a new family with the following characters:

HERONIMIDAE Ward. Moderate sized monostomes with thick, elongate, soft body, slightly flattened, tapering toward both ends. Oral sucker weak, pharynx large, esophagus short or absent; ceca simple, narrow, extending to posterior tip but not united. Vitellaria compact tubular; uterus with four longitudinal regions; genital pore ventral to oral sucker, near anterior tip. Testis tubular, small, copulatory apparatus poorly developed. In lungs of turtles, northern North America.

Among the amphistomes Stunkard (1917) described a very peculiar form in which the oral sucker is subterminal and the acetabulum is divided by a transverse ridge into two pockets; this form which he named *Zygocotyle ceratosa* will not fit into any existing subfamily in the amphistome group, and for it must be made a new subfamily, the Zygocotylinae, which is characterized prominently by features just mentioned, and also by the lobed testis and the absence of a cirrus.

Among the distomes a number of changes seem necessary. The well-known parasite of native American herbivors, which was first named *Distoma magnum* by Bassi in 1875, has been included heretofore in the genus Fasciola, altho it has no distinct anterior cone, set off from the main part of the body, and the vitellaria are confined to the region ventral to the intestinal branches. The suggestion of Odhner that this form should be made a new genus seems thoroly justified by renewed study, and for it I propose the name Fascioloides with the type *Fascioloides magna* (Bassi 1875).

Among the Echinostomes a species from the loon (*Gavia imber*) and from Bonaparte's gull (*Larus philadelphia*) was described by Gilbert (1905) as *Echinostoma spinulosum* Rudolphi; it can not be that species. From Gilbert's description which is good tho not complete I regard it as a member of the genus Stephanoprora Odhner 1902 to which the name *Stephanoprora gilberti* is now given.

Much confusion has been introduced into the family of the Azygiidae by the formation of new genera for forms which are merely extreme types of the genus Azygia. This is a powerfully muscular distome and may be greatly distorted in the process of preservation. Specimens taken from a single host at the same time and preserved in the same way often present marked external differences in size and form. The genera Megadistomum of Leidy and Stafford, Mimodistomum of Leidy, and Hassalius of Goldberger are instances of such extreme specimens that really belong to the single genus Azygia. These genera must accordingly be suppressed. Altho many records were found of the occurrence in North America of the common European species *Azygia lucii* Müller (often wrongly called *A. tereticolle*), the study of a great number of supposed specimens from different localities and hosts has furnished no evidence of its presence here, and I regard the earlier records as erroneous and due to confusion with other native species.

Among those distomes of the Allocreadiidae which have a group of muscular papillae around the oral sucker, Stafford (1904) separated Lander's *Distoma petalosum* as a new genus Acrodactyla from Crepidostomum and Bunodera. The separation is justified, but the name proposed is preoccupied, and I have substituted Acrolichanus with the type species *A. petalosa* (Lander). Stafford states that this form which is common in the intestine of the Lake sturgeon (*Acipenser rubicundus*) in the Great Lakes and St. Lawrence river, is "on the authority of Looss the *D. auriculatum* Wedl of Linton." I am unable to accept this conclusion or the comment of Odhner that *A. petalosa* Lander is a synonym of *A. lintoni* Pratt. By the courtesy of C. H. Lander I have the original drawings of his form, as yet unpublished. A careful comparison of the details in the drawing with the evidence at hand on the other species noted is adequate to establish the distinctness of Lander's type. More data on this group will be published soon.

In 1910 H. L. Osborn established the genus Cryptogonimus to contain a species, *C. chyli,* he had found in the black bass and rock bass from Lake Chatauqua. The attempt to include this form in a systematic treatment of the distomes has necessitated forming a new subfamily for it, characterized as follows:

CRYPTOGONIMINAE Ward. Very small, spinous distomes, of uniform width, with bluntly rounded ends. Oral sucker relatively large and prominent. Ventral sucker double, minute, enclosed in pocket with genital pore between the two parts. Prepharynx, pharynx, and short esophagus present; crura extend to anterior margin of testes. Excretory vesicle Y-shaped, forking at oviduct, anterior branches reach to pharynx. Testes elongate, parallel, dorsal, in posterior third of body; seminal vesicle convoluted, prominent; no cirrus or sac. Ovary ventral, proximate to testes, slightly lobed. Vitellaria scanty, lateral, in central region of body. Uterus with descending ramus on right, slightly coiled, extending to posterior end, ascending ramus returning on left, crossing in front of ovary and passing on right to genital atrium. Eggs small, dark. In alimentary canal of fresh-water fish.

The location of this subfamily is uncertain, but wherever placed it is somewhat isolated. Odhner would include its type genus Cryptogonimus and also Caecincola in the Acanthochasmidae; in that event they both must be regarded as having lost the crown of spines characteristic of the family and are sufficiently distinct to justify the formation of separate subfamilies, at least for the genus Cryptogomimus.

Odhner (1910) has given a very careful analysis of a complex group of distomes which he names the Lepodermatidae. The family is, however, substantially equivalent to Lühe's Plagiorchiidae, and while Odhner's emendations in the description should be accepted it seems wise to retain the earlier name. This very complex group is richly represented in the North American fauna and it is not unlikely that further study will show the need of splitting it up into two or more families. The precise structure of most North American representatives in the group is too poorly known to justify such a step at present. Among the genera included here are the frog lung flukes belonging to Pneumonoeces, recently worked over by Cort (1915). Looss has given such a thoro analysis of generic characters for these forms that one North American group must be separated as a new genus to which the name Pneumobites may be given; *Pn. longiplexus* (Stafford 1902) is the type of the genus. It is characterized by elongate lateral and nearly symmetrical testes, and lobed ovary in contrast with the round, median testes and entire ovary of Pneumonoeces. The forms are larger and thicker bodied than Pneumonoeces and the extra caecal longitudinal folds of the uterus are more pronounced, reaching nearly the length of the body. *Pneumobites breviplexus* belongs also in this new genus.

LARVAL STAGES OF TREMATODA

Some confusion has crept into the literature by virtue of inexact use of the terms employed to designate larval stages of trematodes. The cercaria is the youngest stage in the sexual generation and is produced in a redia or sporocyst; it has ordinarily a period of free existence and a caudal appendage used in locomotion. In exceptional cases the free stage is suppressed and the transfer is passive. The tail may

be lacking and to such forms the name cercariaeum has been given; this term is a convenient group designation to include all cercariae that are at birth tailless and does not properly embrace such as secondarily throw off the tail. The rejection of the tail regularly occurs when the larva encysts and at the same time there is discharged the secretion of the cystogenous glands which in many free cercariae are very conspicuous structures. These two changes mark the transition from the cercaria stage to the young or agamic distome, and after they have occurred the larva may no longer rightly be termed a cercaria. The use of the term encysted cercaria for this stage is confusing and should be discontinued; the collective name Agamodistomum was introduced by Stossich in 1892 for this condition. From this point the change into the adult distome is merely a process of growth in size and differentiation of the sex organs. The agamodistomum stage is often only transitory as when the cercaria introduced into the alimentary canal of a final host rejects the tail and enters at once on the growth period that yields the adult fluke; it may, however be encysted in the flesh of some secondary host in which relatively unchanged it awaits the transfer to a final host before the growth period sets in. There is, however, no morphological distinction between the larvae in these two instances and no call for separate designations. A review of the literature on North American trematodes shows many cases in which agamic distomes have been described as cercariae. The rectification of these errors will aid in the elucidation of the various life histories involved.

MORPHOLOGY OF NEMATODA

Among the Nematoda s. str. it has been the custom to recognize a number of groups of family rank, and no attempt has been made to ascertain the relations of these families to each other or to form of them higher groups. To be sure, some recent workers have exalted the families of earlier workers to superfamilies, and this change seems both advisable and calculated to aid in their more adequate interpretation, but they remain none the less isolated and unrelated subdivisions. I am of the opinion that more precise study of the morphology of these groups will furnish the basis for interpreting their relation to each other. In line with this I wish to call attention briefly to some results of morphological studies which I think serve to clear up the situation in part at least.

In describing nematodes, terms have been used loosely which should have a definite morphological significance, and the confused usage has served to conceal distinctions that exist clearly. One such case concerns the designation of the specialized region surrounding the mouth. All sorts of structures developed at this point are called lips, and various sorts of projections surrounding the buccal orifice are designated

a capsule. If one examines with care the oral armature one finds a number of distinct types of structure, each of which shows various modifications, but in most cases the fundamental type comes out clearly after study if not at first inspection. Three types which are easily distinguished I propose to call lips, jaws, and capsule, giving to each term a definiteness which accords with the condition in the old and well known examples of each and restricting each term to conditions which agree morphologically with the typical case.

True lips are best illustrated by Ascaris (Fig. 1). The anterior end of the body viewed *en face* shows three lobed projections which are varied in form and detail of structure in a manner characteristic of individual species, but which always hold the same relations to the planes of symmetry. One, the large lip, is dorsal, whereas two others, smaller, are ventral. That the large lip is to be interpreted as the fusion of two separate parts may be seen in the size, the two papillae it bears, and in other details of structure. This lip occupies the entire upper (dorsal) semicircle of the oral circumference and the line which separates it from the lower lips conforms to the lateral plane of symmetry. On the other hand the two inferior lips are clearly dextral and sinistral, being divided by a narrow slit that lies in the ventral half of the sagittal plane. These lips work as a three-parted organ, gripping rather weakly small objects that are drawn up between them. The orifice in this case is tripartite with the main axis lateral and the secondary axis extending ventral at right angles from the former.

True jaws are best illustrated by Camallanus (Fig. 3). The oral armature has only two distinct parts, and these are divided along the median line, the slit separating them being dorso-ventral and the parts symmetrical on the right and left. As seen in use this type of structure is distinctly a grasping organ; the parts move against each other with a powerful action and hold with vise-like grip. In the typical case, each part resembles in general appearance the shell of a Pecten and at the outer margin the two fit closely on each other. As the body of the nematode ordinarily lies on its side, such a structure may appear like a capsule, because the dorso-ventral slit is not apparent, but if the head is rotated carefully true jaws are easily distinguished from the true capsule — the type to be described next.

The oral capsule (Fig. 2) is spherical or cup-shaped, as seen best in the strongyles in which it presents great variety in individual details but a clear agreement thruout the group in fundamental features. At the anterior pole the sphere is cut off by a plane at right angles to the long axis of the worm so as to leave a circular orifice which is less frequently oval when the capsule is compressed laterally, but which is not a narrow dorso-ventral slit. The capsule is furthermore possessed of a considerable cavity which may be itself nearly spherical and which

in the higher or modified types carries on its inner wall cutting and piercing organs such as teeth, lancets, etc., as in the various hookworms. The oral margin of the capsule may be papillate, serrate, or ornamented by fine spines as in many sclerostomes.

Perhaps the most invariable and characteristic feature of the oral capsule proper is its rigidity; while the internal features impart to it a definite bilateral character, the external form is unspecialized or at most radial in type. Its function agrees fully with this. The capsule itself is immobile and works as a cupping or sucking organ; and the internal structures move and by piercing or tearing the tissue wall to which the cup is applied, release fluid materials or torn fragments of cellular character which are drawn down the esophagus to serve as nourishment.

Not all oral armatures described for nematodes can be reduced to these three types. In many cases the data are too general and inadequate to permit of any decision as to the fundamental plan of structure represented. More exact study of this region will result in demonstrating the morphological resemblance to the types described above of some mouth parts yet poorly known. Those forms in which the mouth parts are least differentiated are most difficult to interpret and classify. Perhaps it will be necessary to recognize an undifferentiated type with only a few papillae around the oral opening, and it may well be that further knowledge will justify the designation of still other types of oral armature. Meanwhile it is important in the interests of accuracy and clarity to keep at least these three or four types distinct and to examine as many nematodes as possible in order to determine how far they conform to the morphological plans described or depart from them. The exact application of this test in recent work (Ward and Magath, 1917) and in other cases yet unpublished has been of marked service in reaching conclusions as to the true relationships among the Nematoda.

Another morphological factor which deserves emphasis is the structure of the esophagus. The most common type is that seen in the ascarids. It is pronouncedly muscular in type, with the fibers transverse to the long axis of the organ and conspicuous on first examination as cross lines. This esophagus is tripartite in cross section and is a powerful pumping organ. (Fig. 11.)

A type of radically different character is the capillary esophagus long known and exploited as a diagnostic feature in the trichina and whipworm. It consists of a row of cells pierced thruout the entire length by a delicate tube of minute caliber. This tube has evidently no power of changing form or caliber in functioning and is a sucking organ fitted to the ingestion of fluid nourishment exclusively. The various nematodes which possess such a capillary esophagus I have

grouped together in a suborder, the Trichosyringata in contrast with those having a muscular esophagus which form the suborder, Myosyringata.

Among the Myosyringata one may observe some conspicuous modifications of the simple muscular esophagus just described. Possibly the most marked of these is the development of two specialized regions in the canal. The first is purely muscular and conforms to the simple muscular type except that it has no specialized region at the posterior end and is separated by the second region from the chyle stomach or intestine in which the process of digestion actually occurs. There is at most a line, partition, or constriction between the first or muscular region of the esophagus and the second. The latter is not uniform in appearance and may even be muscular in character like the first region. Usually, however, it is granular in appearance rather than striated and has more opaque walls. It terminates posteriorly in the valve or other special apparatus which marks the entrance into the intestine. Its function has not been clearly demonstrated, but it seems not to be a pumping organ.

In forms having a double esophagus various degrees of specialization may be noted. In the simplest case, Haplonema (Ward and Magath, 1917) one can see only a transverse partition (Fig. 12) dividing the esophagus into two regions which are both apparently muscular, but which differ in precise optical appearance so as to indicate functional differences between them. In other cases the distinction in histological character is more marked, but the separation between the two parts is not much more distinct. Finally, in Camallanus a deep constriction divides the anterior region very clearly from the posterior. In such cases the second part is easily overlooked and the description of the first region gives the worm an apparent likeness to the Ascarid type with a simple muscular esophagus. (Figs. 13, 14.)

One finds considerable range in the length, both absolute and relative, of the two regions of the esophagus. In the simplest case yet recognized (Haplonema) the total of both regions is not more than the simple muscular esophagus, but in the forms like Camallanus each region is so long that together both constitute a conspicuous part of the total length of the worm.

The double esophagus is one of the most characteristic features in the structure of nematodes included in the Spiruroidea and its occurrence may be confined to that group exclusively.

Some confusion also exists in description of the structure of the specialized caudal end in the male because of indefiniteness in the use of terms. In many males one finds lateral cuticular expansions about the caudal end which are utilized as grasping organs in copulation. The term bursa has been often used for all these organs; one may,

however, early recognize at least two types that are morphologically distinct, and that may well receive different names. In one type the organ consists of semicircular expansions that include the extreme posterior tip of the body, joining behind it and indicating the median line by a deep notch or furrow where the two folds come together. The organ shows a series of lines or bands that radiate like the sticks of a fan from a basal point on each side, diverging as they approach the periphery of the fold. This organ which I propose to call the bursa is shaped like a shallow cup or saucer and forms a conspicuous sucker-like termination for the caudal end of the male in the true Strongyloidea. (Figs. 8-10.)

The second type often resembles the first in a superficial way, but on more particular examination shows clear differences. The cuticular expansions are narrow linear folds; they extend along the sides of the body for some distance anterior to the caudal tip, but do not reach posteriad beyond the tip. The outer margin of the fold is nearly parallel to the body, but approaches it slowly, since the fold is broadest near its anterior end and tapers to zero between the anus and the posterior tip of the body. These folds possess bands that are generally speaking perpendicular to the long axis of the body, being parallel to each other and not radiating from a common center. These folds are not often broader than the body and may be so very narrow that they are easily overlooked. They may be called alae or wings, and constitute a simpler or less highly specialized type of clasping apparatus than the circular bursate type. The alate type is common and very likely characteristic among the Spiruroidea. (Figs. 6, 7.)

There certainly are other types of cuticular grasping organs among nematodes such as those of the trichina and the funnel-shaped organs of Eustrongylides and Hystrichus. But I have not yet had opportunity to study these personally, and do not desire to do more than mention them here.

Among the Acanthocephala the simple forms which have in the hypoderm and lemnisci only a few giant nuclei constitute a group sufficiently distinct from other types to be ranked as a family which I propose to call the Neoechinorhynchidae with Neoechinorhynchus Stiles and Hassall 1905 as the type genus. The family may be characterized as follows:

NEOECHINORHYNCHIDAE Ward. Acanthocephala with hypoderm consisting of a syncitium in which are six giant nuclei, ordinarily arranged so that five lie in the mid-dorsal line and one in the mid-ventral. One lemniscus contains two giant nuclei and the other only one. These nuclei are usually conspicuous on external examination. The proboscis sheath contains only a single layer of muscles. The cement gland is a compact mass. A neck is lacking. The muscles are weakly developed. The lacunar system is supplied only with simple circular connections.

The type genus of this family has been very fully and accurately described by Van Cleave (1913). He ranked in this genus one species which differs from all others in it in having an elongate proboscis with numerous irregular circles of hooks in the place of the globose proboscis with only three circles of hooks. For this aberrant form I have established the new genus Tanaorhamphus with *T. longirostris* (Van Cleave 1913) as type. The extreme length of the proboscis and the large number of hooks distinguish these forms at sight from those of the genus Neoechinorhynchus. Of other points of difference in structure perhaps the most striking is the constant presence of 16 nuclei in the cement gland of Tanaorhamphus where Neoechinorhynchus has only 8. One notes also that in Tanaorhamphus the hooks of the anterior row are not conspicuously larger than those following, but in Neoechinorhynchus the difference in size is real in all and very marked in most species.

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EXPLANATION OF PLATE

Figs. 1 to 4.—Apical views of anterior end to illustrate different morphological types among Nematode heads.

Fig. 1.—True lips; tripartite arrangement, in which, however, the dorsal lip has two paillae. Ascaris lumbricoides. (After Leuckart.)

Fig. 2.—Oral capsule; orifice an undivided circle surrounded by six equidistant papillae and showing tripartite esophagus inside and below level of capsule. Cylichnostomum coronatum. (After Looss.)

Fig. 3.—True jaws; bilateral type with mouth a dorsi-ventral slit. Camallanus ancylodirus. (Original.)

Fig. 4.—Radial arrangement; six small protuberances of irregular form, often called lips and jaws but evidently not equivalent morphologically to structures shown in Figures 1 and 3. Note however, the four papillae. Protospirura muris. (After Schneider.)

Fig. 5.—True capsule but bent 60° dorsad. Longisection of anterior end to show dorsal (secondary) position of mouth in Ancylostoma duodenale. (From Neumann and Mayer after Brumpt.)

Figs. 6, 7.—Posterior end of male showing true alae in ventral aspect. Papillae or ribs shown in outline only.

Fig. 6.—Alae joined at anterior limit. Physaloptera muris-braziliensis. (From Hall after von Drasche.)

Fig. 7.—Alae narrow and not joined. Spiroptera penihamata. (After von Drasche.)

Figs. 8-10.—Posterior end of male showing true bursa. Rays of bursa shown in outline.

Fig. 8.—Bursa with distinct, well separated lobes, from dorsal aspect. Haemonchus contortus. (After Ransom.)

Fig. 9.—Bursa with slight median notch between lobes. Heligmosomum minutum. (From Hall after von Linstow.)

Fig. 10.—Lobes completely fused along median line to form a single organ. Œsophagostomum columbianum. (After Ransom.)

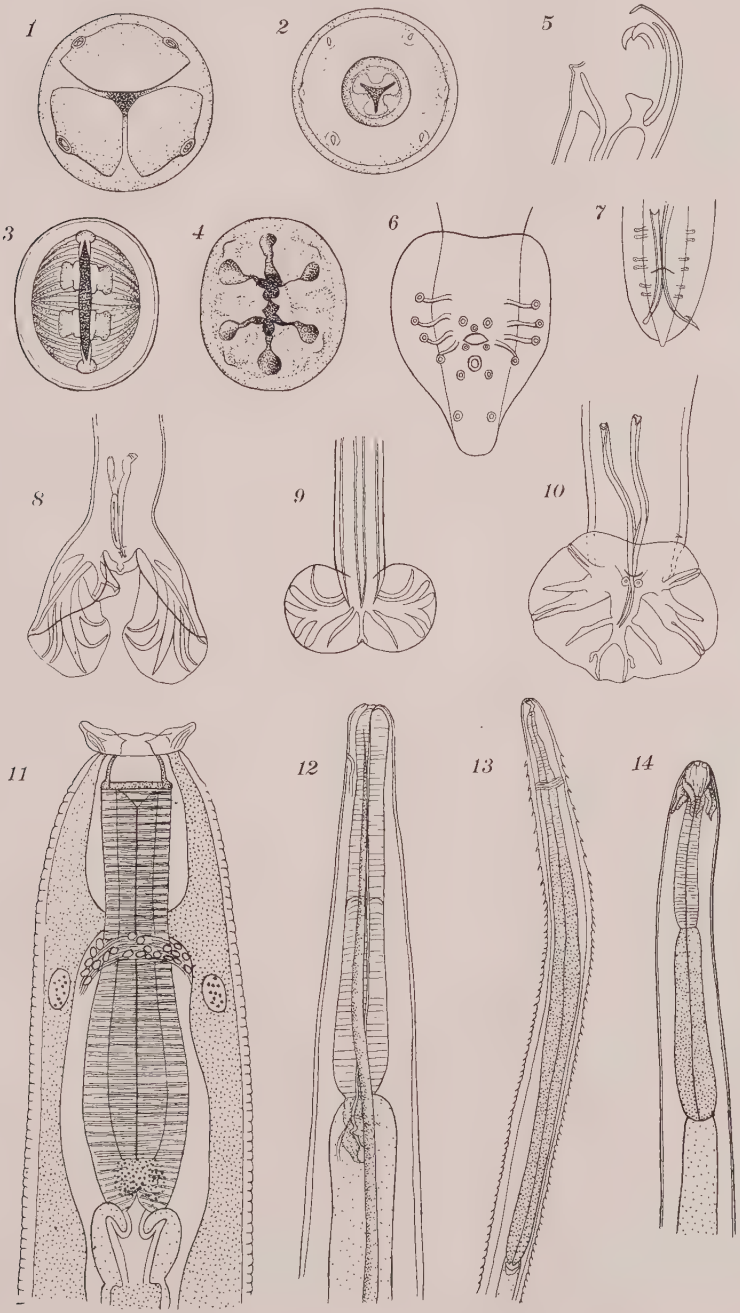
Figs. 11 and 14.—Dorsal views of anterior end to illustrate various types of esophagus among nematodes.

Fig. 11.—Muscular esophagus with single region only. Note, however, granular (glandular?) mass close to posterior end. Cylichnostomum auriculatum. (After Looss.)

Fig. 12.—Muscular esophagus divided by distinct transverse partition near center of length. Haplonema immutatum. (After Ward and Magath.)

Fig. 13.—Esophagus with anterior muscular and posterior granular regions clearly distinguishable but not separated by partition from each other. Spinitectus gracilis. (Original.)

Fig. 14.—Esophagus with two regions, viz. anterior muscular and posterior granular, sharply separated by constriction and transverse partition. Camallanus oxycephalus. (Original.)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ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY

III. ON THE CHLOROMYXUM CLUPEIDAE OF CLUPEA HARENGUS (YOUNG), POMOLOBUS PSEUDOHARENGUS (YOUNG), AND P. AESTIVALIS (YOUNG)

C. W. Hahn

The attention of the writer was drawn to some very common white pseudocysts in the body muscle of the young herring by Dr. Edwin Linton in July, 1910. He had previously (1891) identified the contents of these cysts as myxospores of a myxosporidian. An effort was made at once to trace the life-history of this organism, also to learn its method of infection, the pathological condition induced, and the effect upon the vitality of the host. Several hundred herring, ranging in size from 1½ to 5 or 6 inches have been examined. About 54 per cent of them had buried in the body muscle clusters of myxospores (pseudocysts) large enough to be visible to the unaided eye. These spores are brought to light by cutting the flesh lengthwise of the body on each side of the backbone. As will be evident after reading the following pages, it is certain that a microscopic examination of fish in which no pseudocysts can be seen by ocular examination, would greatly raise the proportion of fish which harbor such parasites. Only small fish under 4 or 5 inches are known to be infected.

The pseudocysts are sometimes as large as a grain of wheat. They are usually white or cream colored, soft or creamy in structure, and spindle shaped, especially when small. Small pseudocysts cannot be distinguished at first sight from worm cysts, but the latter, when pressed with the tip of a scalpel, resist and regain their shape when the pressure is withdrawn. The cysts mash up just like a bit of soft cheese. Usually the pseudocysts lie between the bundles of fibers. Large masses occur in pockets just beneath the integument, which is slightly mounded over them. A pin-prick brings forth a pus-like fluid. The large cysts appear to make their way from deep-seated positions to the surface. A small hole then forms in the integument, through which the mass escapes. No case of the complete discharge of myxospores from such a pore has yet been observed. The process has been observed in its initial stages, and many cases have been observed of worm cysts which were just escaping or had just left pores identical with those just described.

The pseudocysts of this Chloromyxum occur throughout the body musculature. It is very common to find several just at the base of the caudal fin rays. They are also frequent just posterior to the skull and branchial cavity. It is remarkable that a fish can retain life with its flesh so burdened with the cysts, in some cases so abundant that it is impossible to count them. The large ones coalesce and form huge cavities filled with a pus-like fluid. A fish an inch and a half long may contain several hundred pseudocysts and continue for a time to hold its place in a school of several hundred fish against the incessant attack of numerous enemies.

The pseudocysts are composed almost entirely of mature myxospores. When muscle tissue adjacent to the cysts is examined under the microscope, it is found to contain myxospores in masses of all sizes and numerous isolated myxospores or chains of spores between the fibrillae. These aggregations of myxospores vary in size from one spore to two or three times the size of a grain of wheat. The shape is determined by physical conditions. In small cysts it results in long or fat oat-shaped structures.

It is not possible by direct observation to attribute any evil effects upon the host to the presence of these numerous passive pseudocysts. The fish give no visible evidence of inconvenience. But when one takes into consideration the ravages of the trophic stages which must have preceded the harmless myxospores and the toxic substances secreted during the process of sporogenesis, it is very probable that some considerable injury has already been inflicted upon the host before the myxospores develop. My statistical studies prove conclusively that the pseudocysts are in reality more or less injurious.

The trophic stages of the gall-inhabiting species of this genus are known through the researches of Erdman (1910), Auerbach, and Léger (1906). No reference to the multiplicative stages of flesh-inhabiting species has yet been found. The occurrence of myxoplasms in both inter- and intra-cellular positions in muscle tissue has been described by Thélohan (1891, 1893) for *Glugea destruens* Thél. in *Callionymus lyra* and by Gurley (1893) for *Pleistophora typicalis* Gurley in *Cottus scorpius,* and by both Pfeiffer (1891) and Keysselitz (1908) for *M. pfeifferi* in the barbel. The pathological conditions in most of these cases are practically identical to those which I have found in Fundulus. As for details regarding the trophic stages of the parasites themselves, they are scanty and cannot be satisfactorily correlated with life histories as they are now known.

The origin of the multiplicative trophoplasts is still somewhat obscure. It is probable that large myxoplasts like that shown in Figure 15 undergo schizogony and set free the small trophoplasts which

occur so abundantly in newly infected tissues. The smallest of these is about 2μ in diameter. They are very closely scattered in patches throughout the myoplasm of infected muscle fibers, sometimes so close together as to be almost in contact. The fact that the size is not uniform would lead to the conclusion that they multiply by fission, since it is the habit of most myxosporidia to advance from stage to stage simultaneously. But one never meets with couplets in the same cavity in the myoplasm, as would be the case if fission were common.

The distribution of the small and large trophoplasts is very irregular, there being frequent isolated individuals. The muscles of the head region, especially those around the branchial arches and the eye and jaw muscles, are frequently riddled with these parasites. They also occur abundantly in the striated muscle of the digestive tract (the herring has ribbon-shaped striated fibers in the wall of the intestines) and less frequently in the body muscle near the backbone. They are both inter- and intra-cellular (Fig. 8).

When fresh muscle is examined, the trophoplasts appear as almost invisible homogeneous droplets. Stains have no greater tendency to take hold of them than they do the trophoplasts of *M. musculi*. Anilin stains reveal them as white spaces in the myoplasm. Hematein gives to them a homogeneous clouded appearance as shown in Figure 13. The shape in the very small individuals is rounded or ovate. Larger ones are irregular in shape, but in fixed preparations have always an entire contour. Rarely a medium-sized trophoplast has the nucleus faintly stained (Fig. 13).

There is the same evidence that these bodies are not artifacts that has been given for the trophoplasts of *M. musculi*. The appearance of the older stages of the multiplicative trophoplasts is almost identical with that of the young. But the size, shape, and distribution gradually change. Some are evidently motile, judging from their shape (Fig. 13). A few individuals of this type occur in rather isolated positions. The muscle is taken from the body near the backbone. Adjacent tissues are liberally sprinkled with smaller trophoplasts. Older stages than that in Figure 13 are usually still more isolated from the more gegarious young stages. It is probable that the schizogony sets free innumerable multiplicative spores which throughout their growth migrate from atrophied toward normal tissue and become quiescent in the mature schizont (Fig. 15). The latter are very large (sometimes 40 to 50 by 50 to 60μ), and almost spherical in form. They are usually in tissues which are comparatively free from trophoplasts of small size, and also free from evidences of infection. Rarely one can discern a faintly stained material within which is probably the nucleus. Another large body which is almost identical in appearance, but which is not asso-

ciated with the smaller trophoplasts, nor connected with them by any link as yet discovered, is of much greater size. It reaches 890μ in length by 30μ in width. The outline is sharp and the slightly opaque cytoplasm reveals no trace of the internal structure. These bodies lie between the muscle fibers, sometimes in rows. Around and between them is a granular deposit which runs into the masses forming faint partitions. It gives the appearance of a large number of schizonts which have more or less fused. Separate large myxoplasts do occur in exactly similar positions. This form and position in the muscle fibers is also reproduced in a striking way by masses of sporoblasts and myxospores in tissues which lack all of these earlier stages. It is therefore apparent that the large elongated myxoplasts represent aggregations of some kind of migratory trophoplasts. That they do not grow in situ is shown by the uninjured condition of the tissues. They are too large to represent the adult of any single one of the largest myxoplasts without a vigorous consumption of host tissue of which there is negative evidence. The sporoblasts and myxospores occur in chains and smaller groups such as to indicate that there are numerous small clusters of sporoblasts which are not gathered together as in the cases above cited. It is altogether probable that either mechanically or through their own activity, the propagative myxoplasts, having migrated deeper into the tissues of the host, become assembled into larger or smaller groups. In this condition the sporoblast cells are formed and sporulation takes place.

In these two kinds of presporulating cells one evidently has the multiplicative and propagative schizonts. The former represented in Figure 15, is always found in tissues adjacent to the young multiplicative trophoblasts. The latter is never to be found in tissues which contain multiplicative stages, but always in the very presence of sporoblasts and myxospores. The multiplicative stages and schizonts alone are encountered in the muscle of the digestive tract and its vicinity. The propagative schizonts are always in the tissues of the body muscle. These facts prove (1) that the trophoplasts migrate from centers of infection to parts free from previous attack; (2) that the general trend of the migration is from the digestive tract into the body muscle. (3) that the initial infection takes place through the digestive tract. It is probable that this occurs throughout the entire length of the digestive tube, because there is no very marked superiority in the number of myxospore cysts of the anterior body muscle over the posterior region. However, this equality of distribution may be due to transit through the blood vessels.

In the large schizont cysts described above one can occasionally find the contents divided into hundreds of irregular-shaped cells whose

cytoplasm is so clear and structureless that the cell boundaries are almost invisible. They contain conspicuous masses of varying size and shape and intensity of stain. The two upper cells of Figure 1 are almost identical with the above, but were drawn from a group that had been set free from the cyst. These are sporocysts. The deeply stained portion represents the developing myxospores and is not a nucleus as one might suppose.

From the above and further details of sporogenesis which follow, it will be seen that sporoblasts may not necessarily occur free, the large presporulating masses being composed of many assembled myxoplasts of comparatively large size. Within the schizont, there are no doubt many stages of sporogenesis as yet concealed because of inability to stain them. While it is known that the sporocysts arise from some sort of sporoblast or gametoblast cells, the method of origin of said cells is absolutely unknown, whether it be by a continuous process of internal budding or a simultaneous schizogony.

The earliest condition of the spore which I am able to identify with any degree of certainty is shown in Figure 16. It is a sporocyst composed of cytoplasm that is identical in properties to that of the multiplicative trophoplasts. The nuclei do not stain. What appears to be a large nucleus at the center is really the early condition of a myxospore. It is irregular in shape and at first discloses no nuclei. Sometimes these spore fundaments are encountered free from the sporocystplasm as shown above in Figure 16. The rectangular form of the myxospore is assumed later (Fig. 1).

In the homogeneous stainable portion of the sporoblast which later becomes the myxospore, there at first appears a large, more densely staining portion, which, by its behavior, proves to be the nucleus (Fig. 1, upper sporoblast). The nucleus becomes more concentrated (the two lower left-hand cases), and by some method of fission not yet clear, it is divided into as many as nine fragments (Fig. 11). In some cases the sporoblasts contain all of the nine nuclei before there is any evidence of polar capsules. In others the polar capsules appear in the presence of only four or five nuclei (Fig. 1, the right-hand sporoblast). Myxospores with one nucleus opposite the large end of each polar capsule are very common. The others may occupy almost any position in the free periphery of the sporoplasm. In Figure 11 there are two nuclei opposite each polar capsule. Five of these are the generative and wall nuclei which have not yet left their central position. With some stains the polar capsules are conspicuous and the nuclei almost invisible (Fig. 10).

The mature myxospore (Fig. 10) is more or less square with bulging sides. The polar capsules are pointed at the inner end and have a

very short, tapered neck. The myxospores of *C. clupeidae* measure on an average 7μ across from one side to the other. The polar capsules are a trifle over 1μ in diameter and about 2μ in length. I have examined many hundreds of these myxospores and have never discovered any indication of valves in the spore wall.

When compared with the *C. funduli* (Hahn, 1913: 205) it is readily distinguished. The latter is circular when viewed from the polar end and tapers with an incurved outline from the antipolar region to the polar end. The polar capsules are therefore drawn out and curved to correspond to the exterior. In *C. clupeidae* the profile from the polar end is square with rounded corners. It is not drawn out at all on the polar end, but is shaped like a very low conical pyramid with a round base. The profile is, therefore, that of a hemisphere on the polar side and of an oblate spheroid on the antipolar side.

As far as I have been able to discover, there is no Chloromyxum answering to the above description which has previously been described, unless it is *C. quadratum* from the muscles of *Callionymus lyra* (Thélohan, 1895). This is a very similar parasite, though by no means identical. It forms small pseudocysts and masses of myxospores in the muscles. The myxospores occur in small groups or bundles of ten to twelve which are massed together into secondary groups of three to thirty or more. The primary groups of myxospores are probably derived from a single propagative myxoplast. The propagative myxoplasts, having been gathered into masses are thus responsible for the primary and secondary groups above mentioned. No such limitation of myxospore groups has been observed in *C. clupeidae*. Otherwise the conditions of spore formation are apparently the same.

The myxospores of *C. quadratum* are much longer than those of *C. clupeidae*, being 7μ in length along the polar axis and 5μ in diameter, while the myxospore of *C. clupeidae* is 7μ in diameter and not over 5μ in length. *C. quadratum* is deeply incurved on the sides and has a long polar apex with very small polar capsules.

The myxospore of *C. mucronatum* (Gurley, 1893) differs in shape from *C. clupeidae* in a very distinctive way. The profile, as seen from the polar end, is similar to that of the latter, but is circular in outline. The profile from the view at right angles to the polar axis is relatively shorter in *C. clupeidae* than in *C. mucronatum*, otherwise they are very similar. The polar capsules of the latter are relatively a little smaller and shorter. The difference between published figures of the two species may be due to a difference in relative maturity, but *C. mucronatum* is a free-living form from the gall and is polysporous.

The most obvious pathological change which is induced by the *C. clupeidae* is the degeneration of the muscle fibers. As in the inva-

sion of fundulus muscle by *M. musculi,* the early trophic stages cause the myoplasm to hypertrophy. But I have never encountered tissues in the herring that had suffered in a way comparable to those of Fundulus. Parts of the musculature and connective tissue of the intestines of the former are completely disintegrated, while the parasites occur in herring flesh by hundreds of thousands; no gross hypertrophy is ever to be observed. In the muscle of the head the fibers are sometimes riddled with holes containing the parasites. Atrophied muscle fibers are also to be encountered in the body muscle. As such fibers occur in tissues having only multiplicative stages, it is quite certain that the greatest injuries to the body muscle are not caused by the propagative stages. This conclusion is confirmed by the location and habits of the propagative stages themselves.

Because of the pathological condition one finds in the muscle fibers of infected herring, one would expect this disease to be very destructive to the fish. But when caught the fish are in apparent good health. The enormous masses of pseudocysts in the flesh do not inconvenience the locomotion of the herring so far as one can observe by watching schools of young herring as they dart about escaping from their enemies above and below. However, weak and unfit fish would undoubtedly be overtaken by such swift enemies as squid, mackerel, bonito, etc., with which they are constantly beset in the open sea. Those which were severely injured by the multiplicative stages have no doubt already been eliminated from the schools one observes in open water, the survivors having myxospores only. One can only speculate upon the possible mortality of a disease which, having passed through its most virulent stage, leaves considerably over 50 per cent of the survivors infected. Additional observations along this line will appear in a later paper.

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ENDAMOEBA BUCCALIS

II. ITS REACTIONS AND FOOD-TAKING

Nadine Nowlin
University of Kansas

The material for the present paper was collected from the same source as that of the previous study — a single host and a single point of infection, an upper premolar tooth. For details of preparation of material and method of study see Nowlin (1917).

MORPHOLOGY AND BEHAVIOR

The size of the living trophozoite found in smears varies from about 12 to 40μ when in a spherical condition, and the latter may elongate to 80μ. The amoebae most frequently encountered are from 25 to 35μ, constantly forming pseudopodia if it be no more than shoving out a slight rim on one side and withdrawing it. A specimen just out of the mouth is usually active unless it has been chilled or is forming a cyst. It is not necessarily progressing, but can be seen to change shape rapidly by sending out blisters of ectosarc one-third its entire size. One of these rapidly melts into another and will continue to do so unabated for an hour or more if conditions of warmth and moisture are favorable. A group of twenty amoebae massed into a clump were observed vigorously crawling over each other for more than an hour. Food material was the suspected stimulus for such motion, but when they finally separated from a sudden cooling of the slide, no food was visible. They might have exhausted any supply, but more probably they hung together thru positive thigmotaxis.

During progressive movement the endamoeba is an interesting example of the highest development of pseudopodial motion. The animal is elongated to about the proportions of a thumb, and clearly differentiated as to ends. Forward is a clear protrusion of ectoplasm nearly half the length of the body, and rounded out like a bag. At the posterior end is a knob (Fig. 1*a*), which, if torn from an attachment will have little papillae or beads on the periphery (Fig. 1*c*). These threads of attachment enable an amoeba to carry about with it great masses of leukocytes and debris. It exhibits in this most remarkable strength for a one-celled organism. One amoeba can break up a large solid-looking mass of leukocytes by crowding into them. It can load up with a great cargo of cells and tartar which it carries over the slide as long as one has the patience to watch; nor does the load seem to impede its progress.

The very active movements of *Endamoeba buccalis* in smear preparations suggest great possibilities of damage in the gum by purely mechanical processes, tho this is by no means their full capacity for mischief, as will be seen under methods of food-getting.

Reactions to Light and Heat.—Endamoeba buccalis exhibits a much more marked positive thigmotaxis than do free living amoebae. To leave deliberately a clump of leukocytes to which it has been attached and venture into the open requires several attempts. In one case under observation there was a gap a little greater than the width of the endamoeba's body to be crossed before more leukocytes could be reached. The animal extended itself probably twenty times toward the mass, before it relinquished hold, and even then only after it had succeeded by an extraordinary stretch in touching the other side. These parasites have the capacity of stringing a pseudopod to a length of five or six times their body diameter. Attached thus an animal may

Fig. 1.—*Endamoeba buccalis* in movement. For details see text.

wander into a clear field, breaking the thread only when it comes in contact with a solid, or settles down for attachment to the slide. Many amoebae appearing free are found to be thus attached if the light is properly adjusted. Again the pseudopodia may assume a perfect corkscrew appearance, attached to the spherical body of the parasite and moving in various directions like waving tentacles (Fig. 1*d*).

The few experiments performed to test their sensitiveness to light suggest that they have lost this quality. No difference in behavior could be detected in very bright light and in reduced rays. They are very sensitive to heat, however, and where that is combined with light, as is often the case, there is marked reaction, as shown by increase of activity so long as that temperature is kept around body warmth.

No contractile vacuole is present, and only twice in the living specimens have anything like nuclei been observed. One morning every living amoeba in the smears, and many were made, showed a single red spot about the size of the nucleus of stained specimens. The host

had gargled with glycothymoline and that was suspected as the staining agent, but it could never be demonstrated again either by direct application or by gargling. Under one other condition a spot resembling the nucleus showed up uniformly in many living forms which had been kept sealed for six hours under a cover-slip. These endamoebae assumed an encysted form without completing it with the outer wall, lost all their vacuoles, and showed a single dense spot slightly to one side of the center. This may have been a nucleus.

Food and Food Habits.—The adult or mature trophozoite form has from one to twenty food vacuoles. The usual appearance is three or four of these, each about one-fourth the diameter of the amoeba, and many smaller ones. In life, the contents of these large vacuoles are homogeneous spherical masses, having a greenish gray color, and showing only a single mass to a vacuole (Figs. 2*a* and *b*). In stained specimens also the food vacuole contents are solid masses, varying

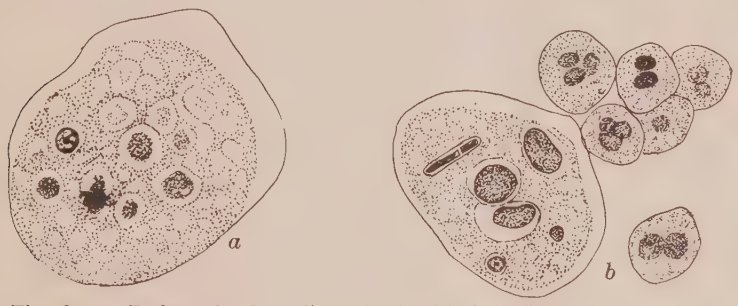

Fig. 2.—*a, Endamoeba buccalis* stained with Dobell to show nucleus. *b,* Stained as above to show food vacuoles and attached leucocytes.

only in intensity of coloring, and showing never any resemblance to leukocytes. They appear more as globules of fluid. No endamoebae under observation during this study, though they have been watched carefully for this, have been seen to ingest leukocytes, red blood cells, as described by other writers, notably Smith and Barrett (1915), or anything solid except bacteria, and these in small numbers. That they never do this, I cannot say definitely, but I believe the following behavior may have been interpreted as ingestion.

The endamoebae frequently attach themselves to leukocytes (Fig. 2*b*). I have seen them creep over the leukocyte and practically surround it until it looked quite like ingestion. A leukocyte will even become sufficiently incorporated to be taken away with the endamoeba for a short time, but if watched sufficiently long the leukocyte is invariably left on the slide, apparently unharmed. I have seen other clear refractive bodies taken in like this and soon discharged, never becoming real food vacuoles.

SUMMARY

I conclude from my observations that *Endamoeba buccalis* absorbs its food mainly, taking in by osmosis the fluids of leukocytes or other media on which it rests, stores these colloidal substances in vacuoles, and by secretion of its own enzymes assimilates these as needed.

The reasons against believing that large food vacuoles are ingested leukocytes may be summarized thus:

(1) There is never but one body to a vacuole, while most leukocytes have one to three nuclei.

(2) There is never any granular area around the vacuolar inclusions, as would be the case if the cytoplasm of a leukocyte were ingested.

(3) Leukocytes have been surrounded by amoebae, but never ingested, according to my observations.

(4) The whole system of vacuoles can vanish from an *Endamoeba buccalis* exposed to unfavorable conditions, sooner than would be possible if these were solid inclusions; moreover, the leukocytes outside the endamoeba are left intact.

This method of food-getting by absorption would explain the shrinkage of gums where *Endamoeba buccalis* is present. I have seen no evidence of their penetrating epithelial cells, but there is abundant evidence that they draw supplies by applying themselves to the surface of tissues and by crowding between them (Fig. 2).

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THE CENTRAL NERVOUS SYSTEM OF THE PARASITIC ISOPOD, GRAPSICEPHON

WILLIAM A. HILTON

Department of Zoology, Pomona College, Claremont, Calif.

Some specimens of the genus Grapsicephon of the Bopyridae were obtained from the gill chambers of the common shore crab of Laguna Beach, *Pachygrapsus crassipes* Rand. One of these was sent to the United States National Museum and there determined to be of the genus here given.

Two specimens were sectioned and mounted in series; one was stained in carmine and one in hematoxylin. Only in the latter specimen was the poorly developed nervous system distinguished easily from the surrounding tissues. No supraesophageal ganglion was found and the ventral chain of ganglia was imperfectly developed. The whole central nervous system does not exceed one millimeter in length, or a little less than one twelfth the length of the animal. A wax reconstruction was made of the central nervous system showing the locations of the cellular areas.

There are at least four ganglia represented in the nervous system, but these are very imperfect and irregular ganglia. Beginning at the cephalic end the ganglion is quite well fused and occupies one third the whole length with no branches for some distance; then there are large irregular branches extending laterad. Next there is a division into something like connectives and other branches extending laterad, although these do not show well in the model, because they seem fused with the other parts. Near the caudal end of the ganglionic mass there are other divisions into connectives and near these, short branches. Altogether, there are six very irregular pairs of lateral branches which could be followed only for a short distance from the central nervous system, and four branches which arise from the caudal end.

The distribution of cells is on the whole much like that of other arthropods. Most of the cells are ventral in position, but irregular masses are seen at places on the dorsal side. The cells in many cases seem but poorly developed; the nuclei in some cases are like those of nerve cells, but most of them appear like poorly preserved material, although the general preservation of all parts of the specimen except this was very good.

In conclusion, it might be said that the animal has a degenerated central nervous system with indications of at least four ventral fused

ganglia. Branches are not perfectly formed and cannot be traced very far. Although there were a few striated muscle fibers in the animals, the movements of the living forms were very slight. If there is a dorsal ganglion it is so poorly differentiated as to be indistinguishable from the other tissues of the animal.

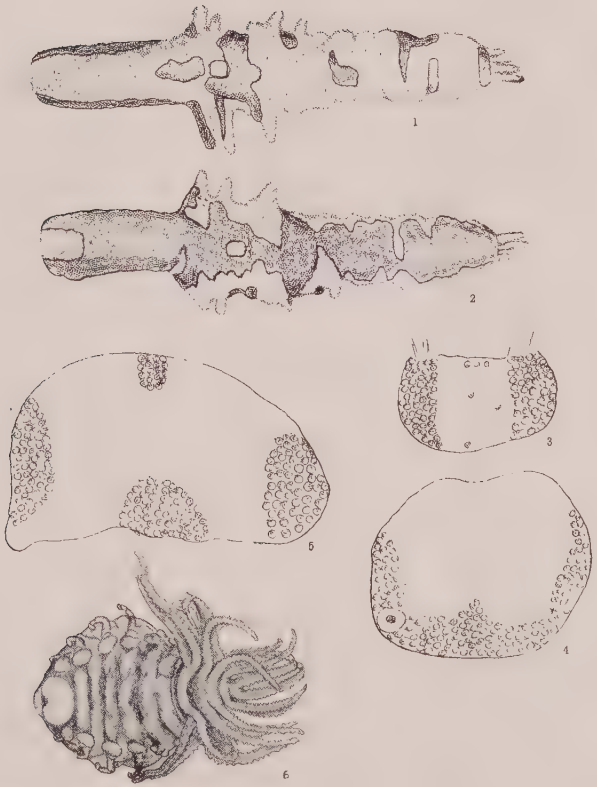
Explanation of Plate

Fig. 1.—Drawing of a model of the nervous system of Grapsicephon, from the dorsal side, showing the cell areas in the more deeply shaded portions. The cephalic end is at the left. × 80.

Fig. 2.—Drawing of a model of the nervous system of Grapsicephon, from the ventral side, showing cell areas by more deeply shaded regions. The cephalic end is at the left.

Figs. 3, 4, and 5.—Sections through various levels of Grapsicephon, central nervous system. The dorsal side is uppermost. × 300.

Fig. 6.—Surface view of the whole body of Grapsicephon. Drawing by Harry Staples. × 3.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INVESTIGATIONS OF THE VALUE OF NITROBENZOL AS A PARASITICIDE WITH NOTES ON ITS USE IN COLLECTING EXTERNAL PARASITES

Wallace L. Chandler

Incident to the recent appearance of an article (Moore, 1916) recommending as a regular procedure the fumigation of animals with *nitrobenzol* for the control of their external parasites, a great deal of interest was aroused in the apparent possibilities offered by the use of this drug in various phases of parasitological work. A number of experiments, both field and laboratory, involving its use were initiated by the Department of Entomology of Cornell University.

At the outset it appeared to those more familiar with the chemical nature and toxic properties of nitrobenzol, that it would be highly desirable to do some definite experimental work to determine the physiological action of this drug on animals when administered by vapor inhalation. There are on record in the Index Medicus (see nitrobenzol, nitrobenzene, oil of mirbane, etc.) a great number of reports of fatal cases of nitrobenzol poisoning in man resulting either from the ingestion of the liquid or absorption through the skin. A few instances of poisoning by inhaling the vapor are also recorded. There have also been done a few careful experiments upon laboratory animals (Filehne, 1878) in which the drug was administered in the liquid form by introducing it into the stomach through a tube and by intravenous injections; and in these experiments symptoms of nitrobenzol poisoning with fatal terminations are described after the administration of even small doses. So far as can be determined, however, no satisfactory experiments are recorded in which the drug was administered by vapor inhalation.

In view of this situation, Dr. M. Dresbach and the writer undertook investigations to determine the action of nitrobenzol upon various animals when such animals were exposed to the vapor of this drug at various temperatures and for various periods of time. The experiments have now been in progress for more than a year and are being conducted at the physiological laboratory of the Cornell University Medical College. The facilities and resources of this laboratory have been placed freely at our disposal. We have also received material assistance and valuable advice from members of the staffs of the

departments of entomology, sanitary chemistry, physical chemistry and histology.

An attempt was made to simulate as far as possible the conditions recommended for the "fumigation of animals to destroy their external parasites." However, the necessity of reducing to a minimum all possible interfering factors, such as impurities in the nitrobenzol, excess of carbonic acid, excess of moisture, and variations in temperature was early recognized. Practically pure nitrobenzol was obtained by redistilling the commercial liquid until a product was obtained which proved experimentally to have a boiling point of 210.9° C. and a freezing point of 5.7° C., the boiling point and freezing point, respectively, of nitrobenzol (Landolt and Börnstein, 1912). Provisions were made in the construction of the apparatus for controlling the other possible interfering factors mentioned.

A detailed description of all the apparatus used will appear in a more technical paper dealing with this subject. In brief, the principal parts of the apparatus were as follows: A paraffin-lined metal tank of 40 cubic feet capacity, so constructed that it could be hermetically sealed with paraffin * and provided with glass windows in top and side through which observations of the temperature of the tank and the animal's condition could be made. In the center of the tank was firmly suspended a wire cage which served to protect the following: A triangular piece of linen, one end of which dipped into a container of nitrobenzol; a thermometer, and a small fan which was kept revolving at a rate sufficient to insure a homogeneous mixture of the air and other gases within the tank. A removable false bottom of wire netting served to keep the animal from contact with its excretions.

The procedure in any single experiment was a follows: The tank was freed from moisture, sufficiently for all practical purposes, by allowing plates of concentrated sulphuric acid to stand in it for a day or so, the tank being sealed. The sulphuric acid was then removed, nitrobenzol introduced into the container, the tank sealed again, the fan started, and a constant temperature maintained. After sufficient time had elapsed to insure complete saturation with nitrobenzol (this was shown experimentally to require several hours), the animal was quickly introduced, the tank sealed, and air saturated with nitrobenzol passed into the tank at a rate sufficient to insure aeration. An outlet was provided so that the air in the tank remained at atmospheric pressure. Observations were recorded as a rule every fifteen minutes.

The results of these experiments, obtained from observations made on a large number of dogs, cats, rabbits, rats, mice, guinea-pigs,

* Paraffin was found to be the best substance for this purpose since it is neither attacked by nor permeable to nitrobenzol.

chickens, pigeons, and frogs, are being compiled for publication in a more extensive paper; but it may not be out of place here to state that in all of the cases observed nitrobenzol has a serious poisonous action when administered by vapor inhalation. The intensity of its action, the type of symptoms produced, and the time elapsing from the moment of fumigation to the first onset of the symptoms vary greatly with different species of animals and even with animals of the same species. The dominant symptoms in all animals seem to be profuse salivation (in dogs, cats, etc., vomiting), diarrhoea, loss of coordination of the voluntary muscles, particularly those of the extremities, and a general loss of muscular tonus. In dogs, guinea-pigs, and poultry muscular tremors were observed. There were also generally present long periods of clonic convulsions ending in tonic convulsions involving the whole body and followed by periods of depression. In rabbits, cats, and rats, depression seemed to be the dominant symptom; while in frogs, depression alone was observed. "In dogs the first symptom shown is vomiting. This is soon followed by loss of muscular coordination. The hind legs are the first usually to be affected; then follow the fore leg muscles, and then those of the neck, jaw, and trunk." (Dresbach and Chandler, 1917.) The animal may recover after about one week,* or may die as a result of respiratory failure following one of the convulsions.

The symptoms of nitrobenzol poisoning may make their appearance during the course of the fumigation, immediately following it, or not until after several days. In dogs four days, and in chickens six days have elapsed between the time of exposure to the vapor and the onset of the symptoms.†

The action of nitrobenzol upon the tissues of animals poisoned by inhaling the vapor of this drug was studied by histological methods. It is interesting to observe that sections through the cerebellum of dogs thus poisoned (fixed in 10 per cent. formalin in saturated aqueous

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*In most of the cases observed the recovery has been complete. Two cases are under observation, however, in which there appear to be permanent cerebellar lesions.

†The apparent degrees of resistance and of susceptibility to the action of nitrobenzol evidenced in animals, even in those of the same species, are extremely interesting. In one experiment three kittens, all of the same litter, were simultaneously fumigated at a temperature of 22° C. for a period of three hours. Two hours after the experiment was begun one of the kittens died. The other two were removed from the tank at the end of three hours and were apparently unharmed, except that digestive functions had been retarded, and never developed any symptoms of nitrobenzol poisoning afterwards. In another experiment, two chickens were fumigated at 23 C. for eight hours. One of them (♂), died shortly after being removed from the tank, while the other (♀), developed symptoms of poisoning only after about six days and the symptoms were then but slight.

solution of corrosive sublimate and stained by Nissl's method) show degenerative changes in Purkinje's cells, while no changes in any of the other cells of the entire central nervous system have been detected. A description of these changes with a discussion as to their significance will be presented in another paper.

The effects of nitrobenzol upon insects, especially fleas and biting lice, parasitic on the animals under observation were studied in connection with all of these experiments. The toxic action of this drug appears to run a more rapid course and to be more intense in the case of the insects observed than in the case of mammals and birds. Beginning shortly after the host was first exposed to the fumes, fleas could be detected crawling excitedly in and out through the hairs, migrating towards the anterior end of the host, apparently as the result of efforts to penetrate more deeply into the hair. In about one-half hour after an experiment was begun it was not an uncommon sight to see the nose of a dog or cat literally swarming with fleas. At 23° C. the activities of the fleas continue for about an hour, when the fleas become stupefied and are easily shaken off by the host. If removed from the vapor after an exposure of one and one-half hours, both the fleas and biting lice of dogs are apparently dead; but while a few of them do not recover, a large percentage of them recover within a short time; in fact, in all cases where conditions of exposure were milder than those corresponding to 26° C. for a period of six hours, a large percentage of both fleas and lice recovered. Likewise, a large percentage of the biting lice of poultry were found to recover after any exposure milder than that corresponding to 27° C. for a period of eight hours. On the other hand, in one experiment an exposure at 25° C. for a period of three hours was sufficient to cause the death of a dog, in another experiment an exposure of five hours at 22° C. caused the death of a dog, while in still another experiment a dog was exposed to the vapor at 20° C. for a period of ten hours and developed no symptoms of nitrobenzol poisoning at all.

In view of the fact that it is impossible to predict just what effect any given condition of exposure to the vapor of nitrobenzol will have on an animal, and the fact that it appears to be impossible to kill either fleas or biting lice by any condition of exposure under that corresponding to 26° C.* for six hours, it is clearly evident that this drug cannot be used with any degree of safety in the "fumigation of animals to destroy their external parasites."† However, since it seems hardly

* It has been suggested that while these insects recover after being removed from the vapor, they may eventually succumb to the action of the drug. Subsequent fumigations of the same animal have failed to prove this to be the case; however, much work is yet needed to be done on this score.

† The action of nitrobenzol upon internal parasites, particularly those inhabiting the blood, is at present being investigated .

probable that one hour's exposure to the vapor of nitrobenzol at temperatures between 20° C. and 25° C. will affect seriously any of the domesticated animals, while fleas and biting lice become stupified after an hour's exposure at the same temperatures and are shaken off by the host in great quantities, it is quite possible that nitrobenzol fumigation may be used to good advantage in collecting specimens of external parasites. The following instances of its use for this purpose may be of interest:

A hen was fumigated at 25° C. for one hour. The hen was apparently unharmed and there were recovered from a sheet of paper previously placed in the bottom of the box more than two hundred specimens of external parasites. The fumigation was repeated on the following day and more than one hundred additional specimens were recovered, making a total of three hundred twenty-six specimens recovered. There were represented in this collection five genera and eight species as follows:

Specimen	Number taken
Goniocotes hologaster	63
Goniocotes gigas	4
Lipeurus heterographus	11
Lipeurus variabilis	2
Menopon pallidum	228
Menopon biseriatum	13
Dermanyssus gallinae	3
Echidnophaga gallinaceus	2
Total	326

In another experiment a hen was fumigated at 26° C. for one and one-half hours. The hen was unharmed and there were recovered more than five hundred specimens of biting lice. Among these were eleven specimens of *Goniocotes gigas*, a species which has been rarely collected in the vicinity of Ithaca.

A young kitten was combed with a fine-toothed comb for the purpose of collecting fleas, but not more than two fleas could be discovered. The kitten was then fumigated at 20° C. for a period of one and one-half hours. In about one-half hour after the experiment was begun the animal's nose was black with highly excited fleas. One-half hour later, the fleas were lying stupefied on the paper in the bottom of the tank. There were recovered eighty-nine specimens of *Ctenocephalus felis* and two specimens of *Trichodectes subrostratus*.

A dog was fumigated at 23° C. for about an hour, and there were recovered a number of *Ctenocephalus*, a great quantity of *Trichodectes latus*, and two specimens of *Haematopinus piliferus*.

In the use of nitrobenzol fumigation for collecting purposes two advantages stand out: (1) Since the parasites become stupefied and are

readily shaken off by the host on a piece of white paper placed in the bottom of the box, great quantities of different species of external parasites may be collected with scarcely any trouble at all; (2) since the parasites are only stupefied and not dead, they may be revived and used for experimental purposes or killed by any method desired.

In the practical use of nitrobenzol fumigation in collecting external parasites the apparatus need not, of course, be so extensive as in experiments to determine the action of this drug on animals, and can be constructed at small cost by any laboratory. It should consist of a wooden or metal box provided with a tightly fitting lid. The size of the box should be determined by the size of the animal to be fumigated (for dogs, cats, and other small animals one of 20 cubic feet capacity will be sufficiently large). The box should be coated inside with paraffin to prevent rust in case metal is used, or to prevent absorption of moisture by the wood in case wood is used. It should have a false removable bottom of wire netting, and should contain a small wire cage which will serve to protect a container for nitrobenzol and the cloth (a triangular piece of cheese-cloth with a 6-inch base will serve) from which the nitrobenzol is evaporated.

The procedure is likewise simple. Preparatory to the fumigation of an animal, the bottom of the box should be covered with clean white paper and the false bottom placed upon this. Nitrobenzol (the commercial product will serve) is then introduced into the container and the box closed. After two or three hours have elapsed, the lid should be partly removed, the animal quickly introduced and the box closed again. After the animal has been exposed to the vapor for from one to one and one-half hours, the box should be opened and the insects, which will be found on the white paper, collected at once and placed in a warm, airy place, in order to insure the recovery of a maximum number.

Operators should be cautioned not to fumigate an animal at a temperature higher than 25° C. or for a longer period than one and one-half hours.

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A MORPHOLOGICAL STUDY OF BOTHRIOCEPHALID CESTODES FROM FISHES*

A. R. Cooper

In this study of North American cestodes of the order Pseudophyllidea the writer has used Lühe's (1902) revision rather than his later (1910) classification, since the family Caryophyllaeidae has not been considered. The order is represented in both marine and freshwater hosts by a number of more or less isolated genera and species, three of the latter of which are new. It has been found necessary to expand the family Diphyllobothriidae Lühe and to erect two new subfamilies, Haplobothriinae and Marsipometrinae, to accommodate the genera Haplobothrium Cooper and Marsipometra gen. nov., respectively.

DIPHYLLOBOTHRIIDAE

The separation of this family from the Ptychobothriidae Lühe only on the basis of the presence or absence of a permanent sac-like enlargement of the uterus (the uterus-sac) and opercula on the eggs would probably now be considered as invalid, were there not other and perhaps more important characters for their differentiation. This is due to the presence of a uterus-sac in the genus Haplobothrium, as has already been emphasized by the writer (1914), and the form of the eggs in Marsipometra.

The only two genera of the subfamily Ligulinae Lühe, namely, Ligula Bloch and Schistocephalus Creplin, are present, each with the single species only that has been accepted by the European workers, particularly Lühe (1910). Both *L. intestinalis* Linnaeus and *Sch. solidus* (O. F. Mueller) are found in the advanced larval condition in the body-cavities of several small teleostean fishes, while the adults live in the intestines of piscivorous birds. Owing to the fact that there are several discrepancies among the existing descriptions of both of these forms, I found it necessary to make a detailed study of their morphology with the view to clearing up the situation.

As already pointed out (Cooper, 1914), the genus Haplobothrium occupies a unique position in the family. What was formerly considered to be the scolex (Fig. 3) is now known to be merely the foremost segment of the secondary strobila. The true scolex (Figs. 1 and 2) is cylindrical or club-shaped and, unlike any other Bothrio-

* Contributions from the Zoological Laboratory of the University of Illinois under the direction of Henry B. Ward, No. 95.

cephalid yet described, has as organs of attachment four eversible proboscides instead of two simple or modified bothria. It thus bears a remarkable resemblance to the scolex of the members of the order Trypanorhyncha, which is emphasized by the fact that each proboscis consists of an eversible portion, a muscular bulb for its activation, and a permanently protruded stump, quite comparable morphologically as well as physiologically with the three divisions of the trypanorhynchid proboscis. The manner of segmentation in this species is also peculiar. As shown in Figure 1, the larva or plerocercoid forms at its posterior end segments which by growth and subdivision in their anterior regions only become, after separation from the primary chain, the secondary strobilas already described (Cooper, 1914a). The genital organs resemble those of the subfamily Diphyllobothriinae Lühe in that they are simple in each proglottis while the openings are all surficial,* ventral and median. The vitelline follicles, as well as the testes, are situated, however, in the medullary parenchyma and within the nerve strands. Other characters which are important in the separation of this (Haplobothriinae) from the other subfamilies are the armament of the cirrus with minute spines and the division of the uterus into a much coiled proximal uterine duct and a large uterus-sac.

As has already been emphasized in the generic diagnosis (Cooper, 1914: 1-2) the nervous system consists of two chief strands situated in the medullary parenchyma outside of the vitelline follicles, uniting in the anterior end of the secondary strobila to form a nerve-ring, and eight collateral strands, four around each main tract, the latter in the jointed portion of the strobila only. In the true scolex, on the other hand, these main tracts are connected by an irregular transverse commissure (Fig. 4), situated among the proboscides, from which branches pass not only to the bulbs, but to the large ganglionic mass (Figs. 2 and 5) behind them. Four large tracts from the latter pass into the bases of the bulbs for the innervation of the retractor muscles of the eversible portions of the proboscides. In the secondary scolex the single large median and two smaller lateral excretory vessels all unite behind the nerve-ring to form a vesicle, but in the primary scolex they are connected not only by numerous large branches among the proboscis-bulbs, but by a single large median frontal loop.

The subfamily Cyathocephalinae Lühe is represented by both genera, Cyathocephalus Kessler and Bothrimonus Duvernoy. The species of the former, found only in *Coregonus clupeiformis* (Mitchill), the common whitefish, resembles the European *C. truncatus* (Pallas) in several points, but in others it is so radically different that it has been

* The word "surficial" has reference to the dorsal and ventral surfaces of the strobila.

considered as new and given the name *Cyathocephalus americanus* sp. nov. The chief nerve strands are connected anteriorly by a number of fine nerve commissures instead of a single one. The opening of the vagina is behind that of the uterus, while none of the genital apertures is surrounded by papillae or sphincter muscles. There is no enlargement of the vas deferens just before entering the cirrus-sac nor connective tissue sac surrounding the whole of the coiled duct as in *C. truncatus*. Furthermore, while the cirrus-sac is not provided with special dorsal retractor muscles, it is surrounded dorsally and laterally by a large mass of peculiar glandular pigmented cells (Fig. 9) the function of which has not yet been determined. The absence of a "connective tissue and muscular sac" surrounding the beginning of the vagina and any such "shell-gland" as described for the European form are also of great importance from a morphological as well as a systematic standpoint.

The generic name Bothrimonus Duvernoy has been taken to include both Bothrimonus and Diplocotyle Krabbe, as contended by Schneider (1902). Of this genus there is present only one species, *B. intermedius* sp. nov., which, while resembling *B. nylandicus* Schneider (1902) in particular, differs from it rather in an aggregate of details than in a limited number of special diagnostic value. The nervous system is, however, radically different in that just behind the scolex each chief strand divides into two sagittally directed branches which are united frontally by a diffuse and not compact commissure.

I have given the name Marsipometra gen. nov. to *Dibothrium hastatum,* briefly described by Linton in 1897, owing to the fact that it cannot be accommodated in any of the existing genera, altho it has several features in common with some of those belonging to the subfamily Triaenophorinae Lühe. The sagittate scolex (Fig. 10), the proglottides, and the segmentation itself are all quite distinct and regular in their nature. Nor is this confined to the external features, for the arrangement of the internal organ-systems is also peculiarly diagrammatic (Fig. 11). The opening of the cirrus and vagina at the bottom of a comparatively capacious genital cloaca (Fig. 13) is marginal and irregularly alternating, while the uterine aperture is surficial, ventral, and on the same level with the genital atrium or very slightly behind it. A conspicuous hermaphroditic duct and a cloacal sphincter are also present. The testes are situated in the medulla between the nerve strands, which are far towards the margins and dorsal to the cirrus-sac and vagina, and are arranged in two lateral fields united ahead of and behind the uterus-sac and central genital ducts. There is no vesicula seminalis immediately proximal to the cirrus-sac, but the receptaculum seminis is comparatively large, rather long, and sharply

separated from the continuation of the vagina, the spermiduct. The ovary is comparable to that of some of the members of the Triaenophorinae in that it is not exactly in the median line, but slightly approaching the margin bearing the genital cloaca. It is also reniform, with tubulolobular wings and a thick, ventrally situated isthmus (Fig. 11). The shell-gland, ahead of the ovary, is likewise not exactly in the median line, but towards the genital cloaca. The vitelline follicles, as shown in Figure 11, are arranged among the body muscles, not in two lateral fields, but continuous from side to side in the anterior and posterior regions of the proglottis. Like the testes they are not continuous from proglottis to proglottis. The uterus-sac is pouched (whence the generic name) and occupies the whole of the medulla dorsoventrally, but not transversely in gravid joints. As in the genus Bothriocephalus, it is developed by the enlargement inwardly of that portion of the duct passing thru the cortical parenchyma. The uterus-opening is towards the margin bearing the genital cloaca, especially in the younger segments; in gravid proglottides it is naturally more median, but never exactly in the median line. Perhaps the most outstanding feature of *Marsipometra hastata* is the fact that the eggs are not provided with opercula, in which particular it is isolated from all other members of the family Diphyllobothriidae. Finally, as regards its systematic position, my study of the anatomy of this species leads me to conclude that not only must it be accommodated in a few genus, but also in a new subfamily the name for which will then be Marsipometrinae subfam. nov.

In the material studied the subfamily Triaenophorinae Lühe was represented by Triaenophorus Rudolphi and Fistulicola Lühe. Altho all of the specimens of the former were larval, two forms from several host species were recognized as being probably the same as the European *T. nodulosus* (Pallas) and *T. robustus* Olsson. Fistulicola is, on the other hand, represented by the single species *F. plicatus* (Rud.) from *Xiphias gladius* L., the swordfish.

PTYCHOBOTHRIIDAE

Lühe's use of the distinction between the two main divisions of the uterus, the uterine duct and the uterus-sac or uterus proper, in the separation of this family from the Diphyllobothriidae, is justified by my study of the developmental relationships of these parts with each other in both Bothricephalus s.str. Lühe and Clestobothrium Lühe. In the species of these two genera the common rudiment of the uterus separates into its two constituents soon after it is formed, which has also been shown (Cooper, 1914 and 1914a) to obtain in the case of *Haplobothrium globuliforme*. Furthermore, in most of the species of

the former genera there is to be seen a method of segmentation of the strobila, which, so far as I am aware, has not yet been described. It consists of a gradual and more or less regular subdivision of a primary segment, which arises from the base of the scolex, into secondary, tertiary, and quaternary subsegments, and even those of the fifth and sixth orders; or into two, four, eight, sixteen, and thirty-two (or more), respectively. Usually, however, the formation of these subsegments does not proceed with the same degree of regularity in all parts of the primary segment, as shown in Figure 18, where the sizes of the dots at the side indicate the values of the subdivisions; there is a sort of dominance of the anterior over the posterior region. This also applies, as can be seen, to the major segments as well, and is on the whole quite comparable to the dominance observed in the experimental regeneration of portions of various planarian worms.

Both of the two well known European species of Bothriocephalus, namely, *B. scorpii* (O. F. Mueller) (=*B. punctatus* Rud., =*B. bipunctatus* Lühe) and *B. claviceps* (Goeze) are recognized and accepted by the writer as American species also, while *Dibothrium manubriforme* Linton and *D. occidentale* Linton are redescribed and placed in the genus Bothriocephalus. *D. laciniatum* Linton and *Bothriocephalus histiophori* Shipley are deleted owing to the fact that they are both considered to be identical with *B. manubriforme*. On the other hand, a fresh-water form, found chiefly in *Stizostedion vitreum* (Mitchill), the wall-eyed pike, is described as new under the name of *Bothriocephalus cuspidatus* sp. nov. It is a medium-sized cestode, up to 180 mm. in length by 2.75 mm. in breadth. The comparatively large scolex (Figs. 14 and 15) is provided with a very prominent terminal disc, deeply notched surficially, and long narrow bothria, quite deep posteriorly. The first segments, while subcuneate in outline and with prominent posterior borders, are almost circular in transection; the middle gradually broaden until they become much broader than long; and the posterior are two to four and a half times wider than long. The genital cloaca is deep and funnel-shaped, and into it the vagina opens close behind the cirrus, the hermaphroditic duct being obscure. The testes on each side of the median genital complex are separated into two unequal fields by the nerve strand (Fig. 16). The cirrus-sac (Fig. 17) is quite large and prominent, being as much as 0.25 mm. in length (depth) and 0.20 mm. in diameter; as shown in Figure 16, it is not exactly median in position. While the ovary is a rather compact organ, the vitelline follicles occupy almost the whole of the cortex, are very numerous (800 to 1000 per proglottis), and are strongly united dorsally and ventrally as well as laterally. The uterine duct is confined to one side of the median line and constantly opposes the

cirrus-sac or coils of the vas deferens, depending on the degree of maturity of the genitalia, both alternating irregularly from side to side. The spherical uterus-sac, on the other hand, occupies when gravid one-third of the transverse diameter of the segment and has its opening in the median line very close to the anterior edge of the latter.

Clestobothrium crassiceps (Rud.), the type and only species of the genus, occurs only in *Merluccius bilinearis* (Mitchill), the silver hake or whiting of the Atlantic coast. Its anatomy has been thoroughly studied by the writer and the somewhat meager descriptions in the European literature greatly augmented, some errors being at the same time corrected.

The other subfamily of this family, the Amphicotylinae Lühe, is represented by the genus Abothrium van Beneden only. Of this genus only *A. rugosum* (Batsch) and *A. crassum* (Bloch) are found in general in marine Gadidae and Salmonidae, respectively. Specimens from *Lota maculosa* LeSueur were considered to belong to the latter species, altho in Europe Lota has been said to harbor the former (vide Lühe, 1910). As a matter of fact, *A. rugosum* presents not a few difficulties as regards its specific identity in Lota in particular, which was keenly felt by the writer in the absence of European material for comparison.

Apart from the species dealt with here the following have also been reported from fishes in America: *Dibothrium* (*Anchistrocephalus*) *microcephalus* (Rud.), *D. aleuterae* Linton, *D. tortum* Linton, *D. cynoscioni* [Linton] Ariola, *D. cordiceps* Leidy, and *D. speciosus* Leidy; but since adult material of none of these was available for study, they must remain for the time being at least as *species inquirendae*. The same may also be said in a certain sense of *Bothrimonus sturionis* Duvernoy 1842, which needs to be reinvestigated from the standpoint of the differentiation of the species of and of the genera Bothrimonus and Diplocotyle (vide supra).

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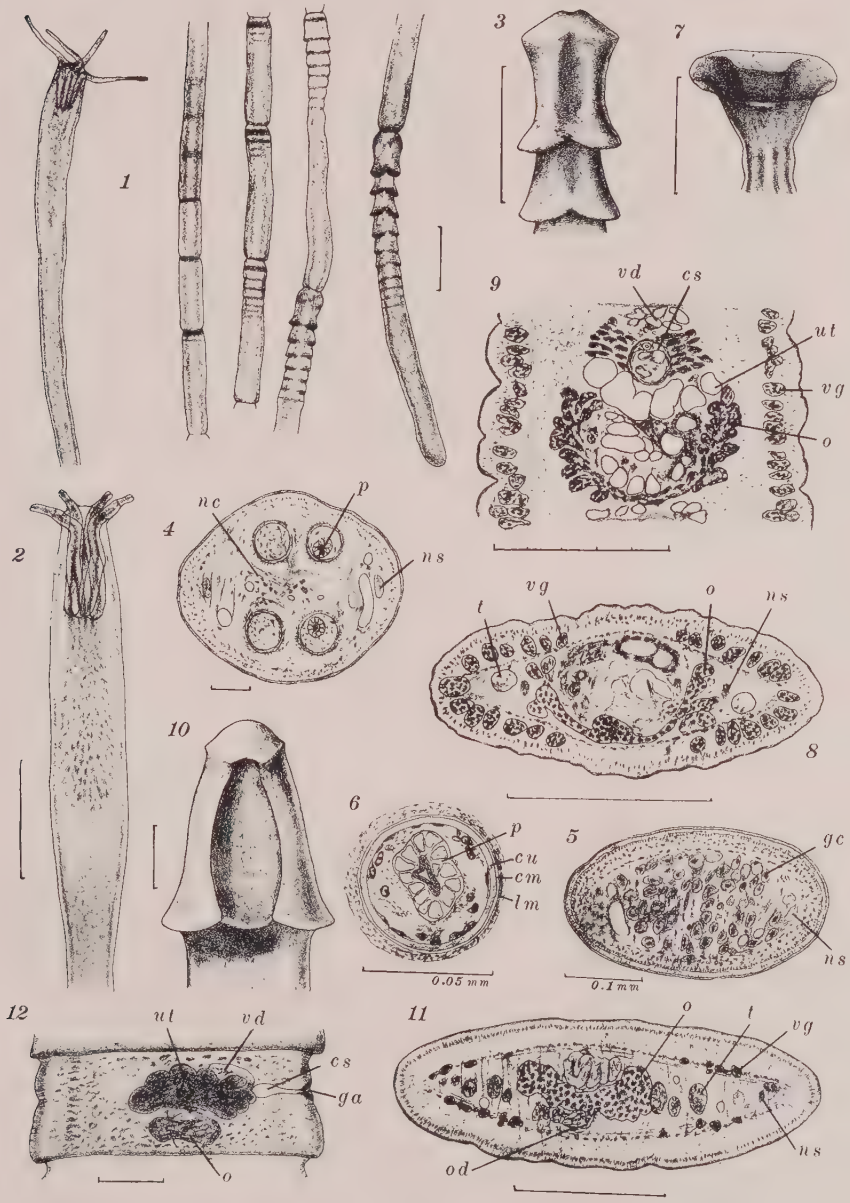
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PLATE 1

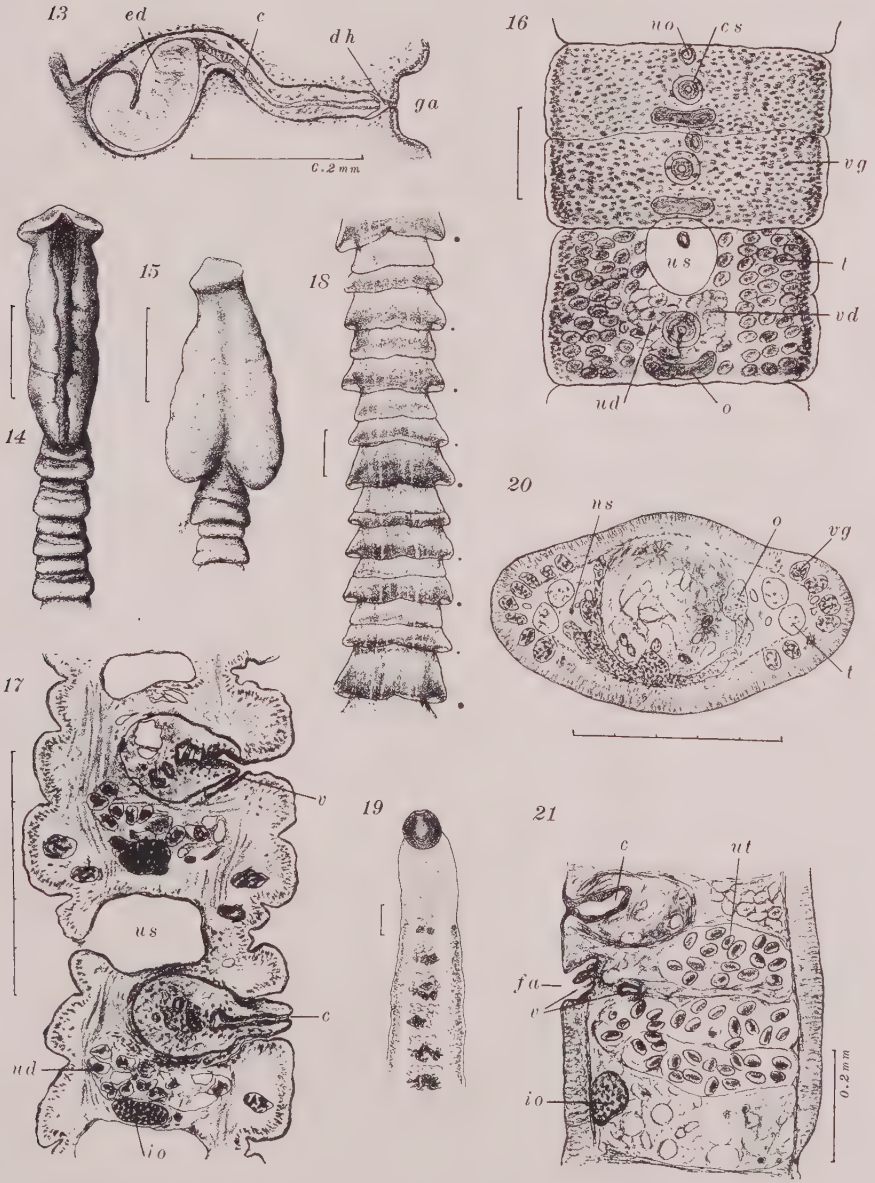
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PLATE II

Explanation of Figures

All drawings were made with the aid of the camera lucida. Unless otherwise labeled, the lines indicating the magnifications are 0.5 mm. in length.

c cirrus
cm circular muscles
cs cirrus-sac
cu cuticula
dh hermaphroditic duct
ed ejaculatory duct
fa female atrium
ga genital atrium
gc ganglion cells
io isthmus of ovary
lm longitudinal muscles
nc nerve commissure
ns nerve strand
o ovary
od oviduct
p proboscis
t testis
ud uterine duct
uo uterus opening
us uterus-sac
ut uterus
v vagina
ud vas deferens
vg vitelline glands

PLATE I.

Fig. 1.—*Haplobothrium globuliforme,* primary strobila, showing formation of secondary strobilas, toto preparation.

Fig. 2.—*Haplobothrium globuliforme,* scolex, toto.

Fig. 3.—*Haplobothrium globuliforme,* secondary scolex, surficial view.

Fig. 4.—*Haplobothrium globuliforme,* transection through scolex.

Fig. 5.—*Haplobothrium globuliforme,* transection through the ganglionic mass.

Fig. 6.—*Haplobothrium globuliforme,* transection through single proboscis bulb.

Fig. 7.—*Cyathocephalus americanus,* scolex, toto.

Fig. 8.—*Cyathocephalus americanus,* transection through ovarian isthmus.

Fig. 9.—*Cyathocephalus americanus,* frontal section of ripe proglottis.

Fig. 10.—*Marsipometra hastata,* scolex, surficial view.

Fig. 11.—*Marsipometra hastata,* transection through ovarian isthmus.

Fig. 12.—*Marsipometra hastata,* toto of ripe proglottis.

PLATE II

Fig. 13.—*Marsipometra hastata,* cirrus-sac from a transection.

Fig. 14.—*Bothriocephalus cuspidatus,* scolex, surficial view.

Fig. 15.—*Bothriocephalus cupidatus,* same, lateral view.

Fig. 16.—*Bothriocephalus cuspidatus,* toto of ripe proglottides, posterior in deeper optical section.

Fig. 17.—*Bothriocephalus cuspidatus,* median sagittal section, composite.

Fig. 18.—*Bothriocephalus manubriformis,* an anterior primary segment; the sizes of the dots at the side indicate the values of the subdivisions.

Fig. 19.—*Bothrimonus intermedius,* toto of anterior end, showing genitalia.

Fig. 20.—*Bothrimonus intermedius,* transection through ovarian isthmus.

Fig. 21.—*Bothrimonus intermedius,* median sagittal section.

NOTES ON KNOWN GREGARINES*

MINNIE WATSON KAMM

The following notes relate to the systematic position of two known species of gregarines. In the one is described and named a species seen but not named by Leidy; in the other is substantiated the determination made by Crawley for a species which he named from two of the three essential characters.

LEIDYANA LEIDYI Kamm *nov. spec.*

[Figures 1, 2, and 3]

Host: *Nyctobates pennsylvanica* deGeer
Habitat: Intestine
Location: Urbana, Illinois, December, 1916

The sporont of this species (Fig. 1) is long and slender, tapering at both ends. The protomerite is only half as wide as the deutomerite at the widest portion; it is slightly constricted at the septum and terminates in a blunt point. The deutomerite is widest in the shoulder region, i. e., a short distance below the septum, and tapers from thence to a long cone blunt at its extremity.

The epimerite (Fig. 3) is a spherical, sessile knob placed at the apex of the protomerite of the cephalont.

The nucleus, obscured in life by dense protoplasm, is spherical and small, situated generally above the median portion of the deutomerite; it contains from one to five large, irregular, deeply-staining karyosomes.

The endocyte of the deutomerite is dense, staining dark and homogeneous, while that of the protomerite is less compact and consists of much larger protoplasmic granules. In transmitted light, the protomerite appears deep tan in color, while the deutomerite is black in the upper portion and gray-black in the lower where the protoplasm is less dense. The epicyte is clear, much thicker in the protomerite, especially at the sides of the septum and in the apical region.

It is apparent that more than one species of gregarine parasitizes this host-beetle. Leidy (1889) describes and illustrates *Asterophora*

* Contributions from the Zoological Laboratory of the University of Illinois under the direction of Henry B. Ward, No. 96.

philica from this host, an attenuated species having for an epimerite "a horizontal circular disc with a round, milled border." The species attains a length of two millimeters, with a ratio of length protomerite to length deutomerite of one to fifteen, and that of width protomerite to width deutomerite of one to one and three-tenths. From an unpublished manuscript of Leidy on gregarines, Crawley (1903a) copied three figures (Pl. III, Figs. 31, 32, and 33), supposedly of the same species, *A. philica,* and taken from the same host-beetle as above. While doubting the authenticity of their relative positions as assigned by Leidy, Crawley does not attempt to further classify them because of the slender evidence, calling all three *A. philica* as done by Leidy.

In my thesis (Watson, 1916) the fact was mentioned (p. 144) that the first of the figures represents undoubtedly the species *A. philica* originally seen as described by Leidy in 1889, for the shape, propor-

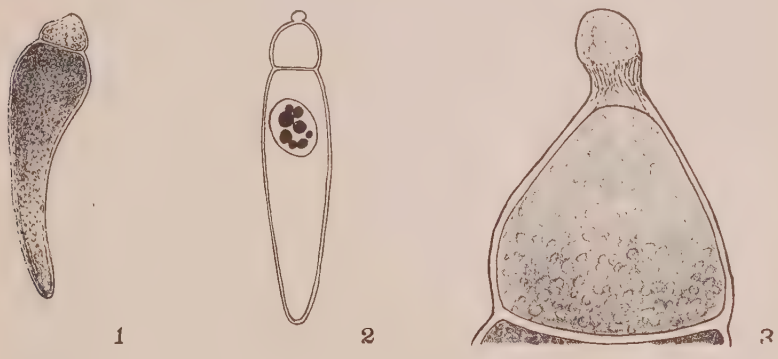

Fig. 1.—Sporont of *Leidyana leidyi* nov. spec.
Fig. 2.—Sporont copied from Crawley (1903a, Pl. III, Fig. 32).
Fig. 3.—Protomerite with epimerite, oil immersion.

tions, and furrowed-disc character of the epimerite agree in the two. It was further mentioned that the second drawing in the Crawley paper (reproduced in this paper as Figure 2) "may or may not be a cephalont of the same species." But, from the data presented above, it is now evident that this cephalont, which has a sessile knobbed epimerite, represents not the same species as the first drawing, but another, the chief differentiating character being the epimerite. In *Asterophora philica,* the epimerite is a horizontal, peripherally milled disc, while, from the abundance of epimerited specimens which I have seen, it a simple spherical knob with no trace of corrugations event under oil immersion. Therefore, the second of the drawings in the Crawley paper and the specimens I have seen correspond and

represent a species which is not *Asterophora philica* Leidy. Below is a table of the contrasting features of the two species:

	A. philica (Leidy 1889)	*Leidyana leidyi* n.sp.
Sporont, maximum length recorded	2000μ	300μ as recorded by Crawley from Leidy's Mss. 550μ from my specimens
Maximum width	150μ	180μ
Ratio LP:TL, maximum	1:15	1:11
Ratio WP:WD., maximum	1:1.3	1:2
Epimerite	A flat horizontal disc with milled border	A smooth, spherical knob

While it is true a single species may undergo decided changes in different environment and even within the same host, yet certain characters are fixed and are used in differentiating species; one of these is the character of the epimerite. It is because of this deviation of the epimerite from the named species that the writer assigns to the specimens seen by Leidy and illustrated by Crawley and to those taken recently a new name.

A new genus has been named to include species living solitary in the sporont stage (until the actual time of cyst-formation), having spherical cysts, numerous spore-ducts, dolioform spores, and a simple globular sessile epimerite, as Leidyana (Watson 1915), differing from the genus Gregarina only in the fact that the animals are solitary instead of biassociative during the sporont stage. In the absence of any data concerning spores and cysts, and because these two genera alone among known gregarines possess simple knobbed epimerites, the present species is placed in that genus as a new species, *Leidyana leidyi*.

A table of measurements of the new specimens in microns follows:

Length protomerite (without epimerite, if present)	20	23	50	50
Length deutomerite	150	187	400	500
Length epimerite (if present)		10		
Width epimerite		10		
Width protomerite	24	40	120	70
Width deutomerite (maximum)	140	75	180	140
Total length sporont	170	220	450	550
Ratio LP:TL	1:8.5	1:9.6	1:9	1:11
Ratio WP:WD	1:1.6	1:1.9	1:1.6	1:2
Ratio LP:TL (Crawley's copy of Leidy's figure of cephalont)	1:6			
Ratio WP:WD (Leidy's figure)	1:1.2			

ACTINOCEPHALUS HARPALI (Crawley)
Gregarina harpali, Crawley, 1903a: 49-50
Actinocephalus harpali, Crawley, 1903b: 637-38

Host: *Harpalus caliginosus* Fabr. (Carabidae).
Determined by Adam Boving
Habitat: Intestine
Location: Atlanta, Georgia, July, 1916

This species, already described, is mentioned here because the epimerite has not heretofore been seen. Crawley has adequately described the sporonts, cysts, and spores, the only stage not seen being that of the cephalont.

The epimerite of the cephalont consists of a small flat disc at the apex of the protomerite and surrounded by a corona of six to nine short, broad, digitiform processes, conforming with that of the type species of the genus in which the species was placed.

This addition to Crawley's description confirms his disposition of the species and completes all the specific characters by which a species is recognized.

That the distribution of the species is rather extended is seen by the fact that the two localities from which it has been taken are Pennsylvania and Georgia.

Additional data is given as to measurements, since the original description mentions only the length as from 225μ to 700μ. Dimensions are in microns.

Length epimerite, if present	20	20	40	
Width epimerite	60	50	50	
Length protomerite, without epimerite	150	170	170	210
Length deutomerite	610	730	880	890
Total length sporont	760	900	1050	1100
Width protomerite	210	170	200	180
Width deutomerite	250	250	250	200
Ratio LP: TL	1:5	1:5.3	1:6	1:5.2
Ratio WP: WD	1:1.2	1:1.4	1:1.2	1:1.1

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FIRST CASE OF LEISHMANIOSIS CUTANEA IN VENEZUELA*

JUAN ITURBE
Member of the National Academy of Medicine of Venezuela
Corresponding Member of the Academy of Medicine of Colombia

AND

EUDORO GONZALEZ

Credit must be accorded to Lindenberg (1909) for having identified the Bauru ulcer of the state of Sao Paolo, Brazil, with the Biskra Button. In substance, the cause of those tegumentary ulcerations is a protozoon of circular or oval shape, of 2 to 4μ in length and 1 to 2μ in width, classified by Vianna (1913) under the denomination of *L. brazilensis,* the specific agent of the leishmaniosis cutanea, a disease known in some parts of Venezuela by the vulgar name of festering wound.

L. brazilensis in preparations colored with Giemsa's stain presents an oval-shaped nucleus of violet tint situated near the anterior extremity; its protoplasm is but slightly affected by the coloring matter and is somewhat bluish.

The kinetonucleus or blepharoplast is located exactly in the line of the lesser diameter of the protozoon. The fundamental character of the Leishmania is the presence in the interior of the protoplasm of a stained band of pale red, situated perpendicularly to the kinetonucleus, which is called *rizoplate.*

The disease has been described in other states of Brazil by Carini (1909), Piraja da Silva (1912), and Matta (1910); in Surinam, by Flu (1911); in Peru by Escomel (1911) and Gastiaburu (1913); in Paraguay by Migone (1913); in Bolivia by Sagarnaga (1912); in Panama by Darling (1911), and in French Guiana by Nattan-Larrier and Heckenrath (1909).

The patient who was the subject of the present discussion, as well as the related microscopic preparations, were submitted to the National Academy of Medicine and studied in our laboratory by Drs. Gorgas, Guiteras, and Carter, members of the Yellow Fever Commission of the Rockefeller Institute, who confirmed our diagnosis.

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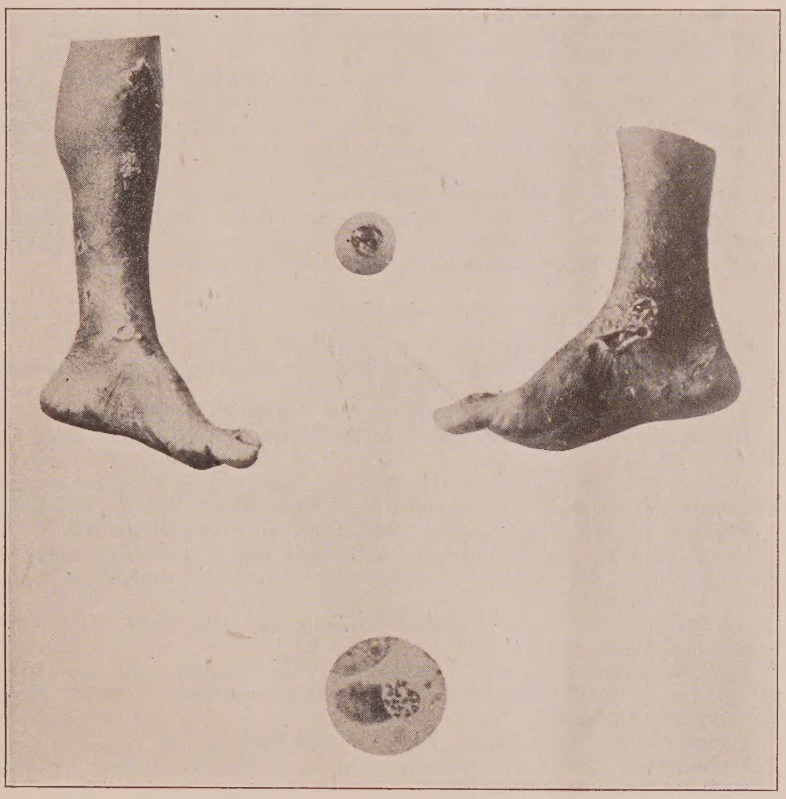
* Paper read at the Second Venezuelan Congress of Medicine, assembled at Maracaibo, 1917. (Contribution from the Laboratory of Dr. Juan Iturbe, Caracas, Venezuela.)

X. X. arrived at our clinic from San Fernando de Apure, where he resides and is engaged in the cattle industry. He is a peasant cattleman of our friend C. E.

He states that two years previously he suffered in both legs various pruriginous acne, hard, violet colored, resisting all medication. These tumors increased in extent until they ulcerated. Some of them healed spontaneously, while others remained in the same state; the ulceration was characterized by hard edges, a cover of black crust, and bad odor.

In the month of August of last year he decided to consult us, having had no improvement from any of the treatments to which he had been submitted.



As may be seen in the photoplate which accompanies this note, the lesions of the skin are localized in both legs. In the right forearm and the knee of the same side movable nodules may be readily observed situated in the subdermic region. During the course of his illness X. X. does not remember to have suffered from fever.

The examination of the blood gave the following result:

Red corpuscles	4,800,000
White corpuscles	10,000
Hemoglobin	73:100

Leukocytal formula

Polynuclear eosinophiles	11 %
Polynuclear basophiles	33.5%
Mononuclear	2.9%
Large lymphocytes	18 %
Small lymphocytes	32 %
Transitional forms	2 %

The Wassermann reaction was ———. The preparations effected with the serosity and the blood of the lesion, previously scraped, colored with Giemsa's stain, showed the presence of a great quantity of *L. brazilensis.*

This case was submitted to the emetic treatment, following the methods of Vianna (1912 and 1914), Carini (1914), and Utra Silva (1915). One month after treatment, the cure was definite.

We employed the emetic of Baiss Brothers in an aqueous solution of 1 per cent. Sterilization is done by filtering cold through a Berkefeld filter. Every two days there will be intravenous injections of 5 c.c. of the solution referred to, until cure is complete. Care should be taken to inject the liquid as slowly as possible, in order to avoid the fits of coughing and muscular pains which are apt to result when the emetic solution is introduced rapidly into the vein.

Lindenberg (1913) has employed also in this disease trixidine (oleaginous emulsion of trioxide of antimony), a substance recommended by Kolle (1913) for the treatment of trypanosomiasis. This has given excellent results.

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NOTE.—Since this paper was put in shape for publication several other cases of Leishmaniosis have been observed in Venezuela. One case treated by intravenous injections of emetin has been sent to me by one of my colleagues in the interior of the country; it has already had a duration of four years.

BOOK REVIEWS

THE KITASATO ARCHIVES OF EXPERIMENTAL MEDICINE. Edited by S. Kitasato, associate editor, K. Shiga. Volume I, number 1; April, 1917. Tokio, Nippon.

The appearance of a new journal in this field would call for comment even if it were not sponsored by the distinguished names of the editor and his associate. In the introduction Professor Kitasato writes of the establishment in Tokio in 1892 of the institute now under government control which bears his name and has made itself a force in the Orient by its work. He refers also to the progress of the Japanese nation in the science of medicine and emphasizes justly the international character of scientific studies, adding in closing the introduction:

"I have been aware that foreigners have wanted to know what the Nippon medical fraternity has been doing in the way of scientific investigation but linguistic difficulties have thus far prevented them from doing so. We hope by the publication of these Archives in English, French and German, to make good this deficiency and to introduce to a wider circle the results of our efforts, and thus bring Nippon medicine to the attention of the world."

The new periodical deserves especial mention in these pages because of the contents of this first number. The introductory article On the Life Cycle of the "Akamushi," Carrier of Nippon River-fever, by Inaka, deals with the structure and development of the red bug or mite by which the disease is transmitted. In his work the author comes to the conclusion that the mite represents a form different from any known adult, and to it Nagayo has given the name Leptotrombidium; the species is then *Leptotrombidium akamushi* (Brumpt).

The third article treats of the ictero-hemorrhagic spirochetosis (Weil's disease) for which Inada in 1915 found the probable cause in *Spirochaeta ictero-haemorrhagiae*. A considerable part of the article is devoted to the organism and to the rôle of rats in its transmission. The last article discusses a new stain for the coloration of protozoans and of blood corpuscles.

The Archives are well printed and splendidly illustrated. The eight plates challenge comparison with any made in other countries.

It is noteworthy that so large a part of this first number is taken up by studies in medical zoology which are marked by their breadth and scientific character. THE JOURNAL extends its congratulations to the editors of the new Archives with best wishes for its continued success as a worthy representative of medical research in a great nation.

Kobayashi has published in the Mitteilungen der medizinischen Fachschule zu Keijo an extended study on the life-history and morphology of the liver distome (*Clonorchis sinensis*). In all twelve fishes have been found to harbor the encysted distome and are the source of human infection. From 23 to 26 days are required for the attainment of complete maturity in the final host; during this period spines appear and then disappear, a fact which has led to a difference in the descriptions of the worm given by various authors. All the Japanese liver distomes are really a single species and not as claimed by Looss two forms, one large and one small with differences in structure and range. The work contains a mass of detail to support these and other findings, and is illustrated by five fine plates.